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Inkjet Printing and Personalised Medicine:
Possibilities and Practicalities

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Abstract

Introduction

Inkjet printing has recently become an interesting technology for the manufacture of drug delivery systems and this has coincided with a burgeoning interest in the personalisation of medicine. Although inkjet printing is an established technology it has not historically been heavily researched in regards to applications within the pharmaceutical industry, in part due to a focus on mass production and markets. As niche markets and individual needs are given more attention and are more economically and therapeutically pertinent, the suitability of inkjet printing as a means of manufacture of several drug delivery systems has been considered and researched within the last decade.

Objective

The objective of this study was to investigate the suitability of inkjet printing as a novel manufacturing technology. This was assessed through giving focus to the printing of proteins and poorly soluble drugs, the coating of microneedles, and the parameters required for successful printing.

Methods

Ten studies were selected to answer the questions posed from extensive searches within the databases PubMed, Scopus, and Web of Science. Searches were performed with filters and specific MeSH terms. Information was also gathered from official sites and a book.

Results

Experimental studies showed success in the use of inkjet printing for manufacturing flexible oral doses with a range of poorly soluble drugs and proteins. Varied release profiles and doses were achieved. Microneedles were successfully coated with inks containing protein or poorly soluble drugs. Printed oral doses and coated microneedles were shown to have suitable release rates.

Discussion

Inkjet printing was shown to be a successful and suitable technology for the printing of oral dosage forms and allowed for a great degree of flexibility allowing for the personalisation of medication. The coating of microneedles was also shown to be viable and could allow for novel administration methods of proteins. Inkjet printing allows for a reduction of waste and the printing of a wide range of active pharmaceutical ingredients - making the technology economically interesting. Ink formulation is an aspect of great importance that needs further research, as does the application of GMP, dose characterisation, and the ancillary equipment used.

Conclusion

Whilst inkjet printing is an attractive technology for the production of personalised medication, the technology is still in its infancy and is currently only suited to experimental research. Once the technology is more established and standardised it may prove an effective method for production of small scale batches of medicine intended for individuals or small groups. This production is likely to be suitable for on-site production at hospitals or even pharmacies.

Keywords: Inkjet printing; Piezoelectric; Personalised medicine; Drug delivery; Microneedles

Contents

Abbreviations

AFM: Atomic Force Microscopy

ANOVA: Analysis of Variance

API: Active Pharmaceutical Ingredient

CD: Circular Dichroism Spectroscopy

CIJP: Continuous inkjet printing

DMAc: Dimethylacetamide

DOD: Drop-on-Demand

DSC: Differential Scanning Calorimetry

EMA: European Medicines Agency

GMP: Good Manufacturing Practice

HPLC: High-Performance Liquid Chromatography

HTS: High Throughput Screening

ICH: The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals

IJP: Inkjet Printing

NIR-CI: Near-Infrared Chemical Imaging

PCS: Photon Correlation Spectroscopy

PEG: Polyethylene Glycol

PET: Polyethylene Terephthalate

PG: Propylene Glycol

PLGA: Paclitaxel-Loaded Poly(lactic-co-glycolic acid)

POX: Poly(2-ethyl-2-oxazoline)

PRX: Piroxicam

PTX: Paclitaxel

PVP: Polyvinyl Pyrrolidone

SEM-EDX: Energy Dispersive X-ray Spectroscopy

SOL: Polyvinyl Caprolactame-polyvinyl Acetate-polyethylene Glycol

SPM: Scanning Probe Microscopy

ToF-SIMs: Time-of-Flight Secondary Ion Mass Spectroscopy

1. Introduction

Inkjet printing (IJP) is a newly established technology that has many potential applications within biotechnology and pharmaceutics. However, the techniques used are still being refined and explored, despite the technology having roots in the mid-1900s. It is an attractive technology as it increases efficiency, individualisation, and preciseness whilst limiting wastage. The technology allows for better and more individualised design that can be adapted to the needs of the patient or drug administrator. Inkjet printing can prove to be an invaluable tool from drug development to treatment and maintaining compliance due to the degree of flexibility that the technology allows. Inkjet printing technology can broadly be categorised as utilising two methods - a continuous stream of ink or precise drops produced on demand. Continuous inkjet printing (CIJP and drop-on-demand (DOD) printing have been the most explored methods for printing. DOD is the current dominant method as it allows for higher precision and quality as opposed to continuous inkjet printing, which involves a continuous stream of substrate. DOD printing is often preferred within the pharmaceutical industry due to the higher degree of customisation and precision which can be achieved (1 - 4).

The desire for personalised and customised medicine can be seen as a result of increased genetic and genomic knowledge and the application of that to explore how individual variance affects standard pharmaceutical treatments. Many mass produced drugs available on the international market have gone through clinical trials where a large proportion of participants are initially male and within a certain age range. Later studies often include a more varied test group but biases in gender, age or ethnic background may still persist. This obviously creates the potential for complication when choosing appropriate dosage for the geriatric population or outlying individuals. Mass produced medications are often available in discrete concentrations and many are not suitable for splitting due to format (capsules, transdermal patches etc.) or due to a failure to ensure an even concentration of active pharmaceutical ingredient throughout a tablet (1 - 3). An ideal solution is not only a tailored dose but also a tailored delivery system for a specific patient.

1.1 Continuous Inkjet Printing
Continuous inkjet printing, as mentioned, utilises a continuous stream of ink which is deposited on a chosen medium at high speeds. The stream of ink is broken up into separate and distinct drops by exploiting the natural tendency of streams of liquids to form drops or particles with set spacing dependent on specific wavelengths of surface distortion and the nozzle radius. The breakup of a liquid stream into droplets with specific spacing is referred to as Plateau-Rayleigh instability and is tied to surface tension and minimisation of surface area. This is made use of in CIJP by where a piezoelectric transducer modulates the flow of ink through the nozzle at a desired frequency to form drops which are suitably spaced, see figure 1. Once the drops are formed, they need to be guided to the correct target area; this is achieved through the use of an electrically charged element resulting in a certain drops gaining a charge. Drops that are not charged are recirculated and the liquid is reused. Charged drops pass through an electric field between deflector plates and onto the determined position on the substrate. The drop reaches the determined position by a combination of charge and speed as determining factors. As a continuous stream of ink is used at a high speed, the integrity of fine details may be compromised. This aspect of CIJP makes it more suited to being applied in packaging rather than printing pharmaceutical inks - which may require specific geometric patterns to be printed at a small scale (1,2).
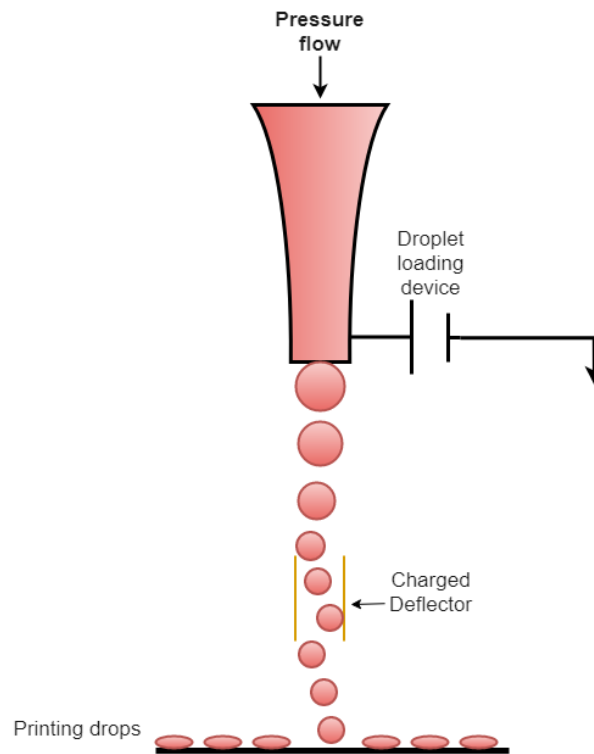
Figure 1. Schematic over mechanisms behind continuous inkjet printing. Drops are formed by a transducer or droplet loading device modulating the flow of ink through the nozzle. Drops are then guided into position onto the substrate by a charged element. Adapted from (5)

1.2 Drop on Demand Inkjet Printing

Current DOD technology can largely be split into technologies utilising heat (thermal drop on demand) and those utilising piezoelectricity (piezoelectric drop on demand). Thermal inkjet printing is based upon heating the "ink" solution via a film resistor element. The heater is activated by means of an electric pulse and the resulting vapour bubble forces the solution out of the nozzle for deposition. Thermal inkjet printing in experimental studies has largely been performed with commercial printers used for traditional paper printing, which may be modified. Piezoelectric inkjet printing makes use of a piezoelectric material and its subsequent deformation to produce pressure waves as the piezoelectric material expands and forces a droplet to be expulsed via the nozzle. The piezoelectric effect exploits the potential differential of opposing faces of a piezoelectric material, a differential that results from applying pressure to the material. The result is an electric charge. The reverse piezoelectric effect arises when an electric charge is applied to a piezoelectric material and the consequent potential differential results in the material deforming (1, 4). Piezoelectric materials are often nonconducting crystals but ceramics and organic materials such as polymers, and biological materials can also exhibit piezoelectric properties. Ideal drop deposition involves the formation of a ligament or tail of liquid being expelled from the nozzle and condensing into a drop shortly before hitting the substrate, as illustrated in figure 2. Should a drop form prior to this due to lower than ideal viscosity, satellite drops are formed. This results in a lower quality print and may affect the desired effect of the printed product.

 Acoustic and electrostatic based inkjet technologies are alternatives though the technologies are still being developed and thus applications of the technologies may not be as clear or studied.
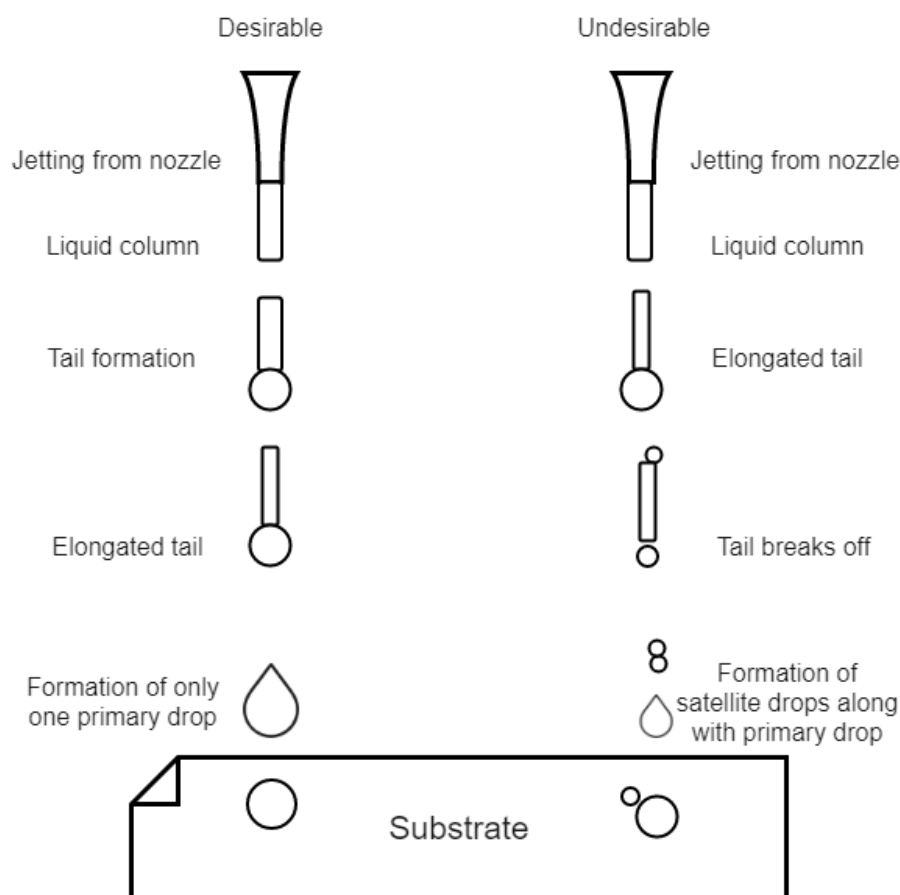
Figure 2. Schematic of ideal drop formation during inkjet printing. An ideal Z-number is desirable to ensure the formation of a ligament or elongated tail forming during expulsion from nozzle. This ensures a single drop. If the Z-number is less than ideal, the ligament will break prematurely and satellite drops will form alongside the main droplet. Adapted from (8)

1.3 Potential Areas of Application

Potential applications for piezoelectric and thermal inkjet printing have a large scope within drug formulation and administration as the technology opens new possibilities for active pharmaceutical ingredients, individualisation, utilisation and increasing compliance. The technology is more established and refined than acoustic inkjet printing and thus makes it a more attractive method with which to explore as a method of medicine production. Current research into IJP as a tool within pharmaceutical discovery and production has been focused on smaller scale production. One major aspect that needs to be taken into consideration when developing a carrier formula for an active pharmaceutical ingredient (API) is how well the formulation performs during printing - it is not enough that it is chemically suited to the API. The carrier fluid must have a surface tension great enough that the liquid does not drip without stimulus and a viscosity low enough to inhibit satellite drop formation - smaller drops surrounding the main drop. The rheology of the fluid must be taken into account and by calculating the Ohnesorge number and/or Z-number, the suitability of a carrier fluid can be judged. The Ohnesorge number relates to the fluid's viscosity, surface tension and inertia whilst the Z-number is the inverse of the Ohnesorge number. The suitability of a carrier fluid can then be evaluated by the Z-number; experimental studies have shown that the optimal range is between 4 and 14, which results in optimal drop formation and deposition. By manipulating the pattern of deposition onto the substrate, the release profile of the formulation can be altered and tailored to a specific treatment regime or individual (2, 3, 5). Due to the nature of IJP equipment, it is especially suited to production on an intimate scale so that a bespoke dosage, formulation and delivery method may be produced for a patient at their local hospital or even pharmacy. IJP technology has already shown

potential for mid-scale production in a factory setting due to the appropriation of the technology in other areas of industry (1, 2, 3).

Inkjet printing is a technology that can prove to be a method that can modernise and streamline many conventional administration methods. It can also allow for the production of novel methods of administering established APIs as some traditional methods such as tablets/capsules and needles can prove problematic to certain populations due to fear or difficulties in administering. The use of hypodermic needles as a conventional administration method for vaccines and drug delivery intradermally and intramuscularly even poses certain problems logistically in addition to limiting the selection of APIs available. Traditional vaccines often require a stable and relatively low temperature from production to just before injection. This can pose an issue when a so-called " unbroken cold-chain" is required due to economic or resource limitations. This is primarily a problem within the developing and the least developed countries - countries that are often in need of vaccines and already face political, economic or logistical issues regarding prevention of infectious diseases. Microneedles have several advantages over traditional hypodermic needles in such situations as they do not require a controlled cold chain and by virtue of that can be transported far more easily. As noted, without the requirement of a cold-chain, more API can be taken into consideration. Bulky packaging is minimised and biohazardous waste is limited - especially in the case of dissolvable microneedles. The latter is especially advantageous due to potentially limited access to correct disposal methods for sharp and/or biohazardous waste. Due to the less invasive and technical nature of microneedles and their application, the personnel performing vaccinations do not need to be as highly trained in comparison to those administering vaccines via hypodermic needles. As IJP can deposit an API - including proteins - onto a variety of surfaces, it can be considered an interesting and viable option for microneedle fabrication. Microneedles can be utilised as an intradermal/percutaneous drug delivery method, allowing for an increased immune response to a lower dose. This is possible as the layers of the skin penetrated by microneedles contain significantly higher levels of dendritic cells than muscles - where vaccines are usually delivered. As a result, the immune response triggered by microneedle vaccination is higher than by hypodermic vaccination, despite only needing a fraction of the vaccine. Economic aspects of vaccinations and the logistics are often factors that lead to ineffective or incomplete vaccinations of populations, especially in developing and the least developed countries. This contributes to the spread and high death rates of certain infectious diseases. Advances within microfabrication technology has allowed for novel approaches to traditional delivery techniques such as microneedles and their formulation and design.

Tablets are potentially the most common method for drug administration and can be formulated to have direct release or controlled release of an API dependant on the treatment needs. Enteric coating can be applied to tablets or capsules to control the place of release and subsequent uptake. This allows for a far larger degree of control in maintaining therapeutic plasma concentrations but due to a lack of dose variation in many controlled release formulas, the concentrations may rise above or below the therapeutic index in individuals. An extreme consequence of this can be a review of the formulations suitability on the market, as is the case with Alvedon 665 mg®. This can be described as a symptom of the wider need for more individualised dosage options and thus a new strategy for the production of tablets or other oral delivery methods. Oral delivery is a highly popular drug delivery system in Europe and the USA and so it is sensible to invest in the development of either smaller scale production of individually tailored doses or more widely produced delivery systems with a high degree of customisation.

2. Objective

The objective of this literature study is to review recent developments in inkjet technology as a suitable method of producing personalised medication. The study focused on the printing of poorly soluble drugs and proteins and microneedle technology as a means of drug delivery. The study seeks to answer: Are proteins and poorly soluble drugs able to be printed? Is inkjet printing technology suitable for coating microneedles? What are the main parameters required for successful printing of an active pharmaceutical ingredient (API)?

3. Method

The methods used in this study encompasses targeted literature searches focused upon inkjet printing and microneedles in databases such as Pubmed, Scopus, and Web of Science. The searches were refined by only accepting articles published within the fields of biochemistry, medicine, pharmacology, toxicology and pharmaceutics, and immunology and microbiology. Articles and studies in Swedish and English were accepted within the initial search but only articles in English were available and relevant. Search words included "inkjet printing" which was combined with the following: "personalisation/personalization" combined with "medicine", "drug eluting stent" "microneedle", "implant", "protein", "soluble". No limit was set on the age of the study or article due to the technology being relatively new. Peer-reviewed published articles were chosen from established and well-regarded scientific journals during the winter and spring of 2018. Examples of search terms and filters used in PubMed can be viewed in table 1. The studies chosen as part of the results were chosen due to their relevance in answering the questions posed. Selection from specific journals and the application of filters such as was necessary in part due to the volume of results but also to maintain integrity and quality of accessed information. A search on PubMed of the term "inkjet printing" returns over 900 results. However, using a combination of search and MeSH terms allowed for a far smaller volume of results. PubMed filters were also applied for the same reason. Other sources of information were accessed from the webpages of international bodies such as the European Medicines Agency and The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. These sources of information were deemed as trustworthy due to belonging to and being managed by international regulatory bodies.

Table 1. Search results and terms from literature searches in PubMed

Date	Search Terms	Filters	Results	Sources
180113	(technology, pharmaceutical[MeSH Terms]) AND inkjet	English; Human; Review	64	1, 2, 24, 26 - 28
180216	(technology, pharmaceutical[MeSH Terms]) AND inkjet AND personalised	English; Human; Review	20	6, 16

180304	(technology, pharmaceutical[MeSH Terms]) AND inkjet AND protein	English; Human	9	36, 37
180216	(("technology, pharmaceutical"[MeSH Terms]) AND dosage forms[MeSH Terms]) AND ink jet	English; Human	10	14, 17
180205	((technology, pharmaceutic[MeSH Terms]) AND precision medicine[MeSH Terms]) AND drug delivery system[MeSH Terms]	English	24	33

This review could have been expanded to also consider studies on 3D-printing - a related technology which may also be a prime candidate for increasing the personalisation of medication. The scope could additionally have been broadened to consider the issues within selected populations such as the paediatric and geriatric that arise due to poor dosage options and poor compliance. This would have allowed for a clearer view of the need for personalised medication in practice. A deeper exploration into the ethics of personalised medicine would have allowed for a more humanistic view on the pitfalls and benefits involved. A further consideration could have been a comparison of current APIs that have characteristics which make them theoretically suitable for use in the context of ink jetted personalised medications. This review focused on piezoelectric inkjet printing as it is the most established technology but this leaves the review blind to potential benefits of other ink jet technologies.

4. Results

4.1 Nanosuspensions as advanced printing ink for accurate dosing of poorly soluble drugs in personalized medicines. (7)

Folic acid, a poorly soluble drug was formulated as a nanosuspension and experimentally tested for reproducibility, printability via ink jet, storage stability and dissolution.

A 10% (w/w) folic acid suspension was produced using a mortar and pestle and an aqueous solution of 3% Tween-20. A nanosuspension was then created by use of a Panda 2 K, NS1001L Spezial homogeniser and pressure. The particle size of the nanosuspension and suspension was determined by laser diffractometry (LD) and photon correlation spectroscopy (PCS). Stability of the nanosuspension was determined by measuring the Zeta potential. Physical stability was determined by constant agitation (150 rpm) at 25 ± 2 °C and without agitation at temperatures 5 ± 3 °C, 15 ± 2 °C and 25 ± 2 °C and measurement of particle size by photon correlation spectroscopy and laser diffractometry. Chemical stability was evaluated via high-performance liquid chromatography (HPLC) by measuring the content of folic acid present in the suspension on day 1 (day of production) and day 30. X-ray diffraction was used to study the crystalline character of the suspension and nanosuspension, with the addition of carob gum to enable analysis without the requirement of drying.

A piezoelectric inkjet printer, Microdrop MD-K-140, was used for printing and a nozzle aperture size of 100 μm. A dissolution test was carried out under sink conditions using a Dissolution Pharma Test Type PTW S3C. The dissolved amount of folic acid was determined by HPLC and the experiment was executed in triplicate and the Wilcoxon test (α = 5%) was used for statistical analysis.

Analysis of nanosuspension particle size by LD and PCS showed a homogeneous particle size and narrow range of size distribution that fell within the specified boundary of a particle size below 5 μm for printability. Three batches were tested and to evaluate the reproducibility the relative standard deviation was calculated. All measured parameters had a relative standard deviation of < 10% showing a high level of reproducibility.

Crystalline state evaluated by X-ray diffraction showed no change in peak location in diffractograms indicating no change in crystalline structure between the suspension and nanosuspension. The peak location being singular suggested a lack of polymorphism and was in agreement with previous characterisation of folic acid crystal structure in the literature.

The suspension and nanosuspension both showed good physical and chemical stability. After storage at set temperatures and agitation, minor changes of the size and behaviour of larger particles was shown by LD measurement. The changes were minor enough not to impact printability. No changes were detected in the mean particle size by PCS. A high zeta potential was tested for the nanosuspension, indicating a high level of storage stability. The content of folic acid measured on day 1 and day 30 was considered to be constant. Analysis of folic acid suspension saturation showed a saturation solubility of 8.96 ± 1.94 μg/ml. When the particle size of folic acid was further reduced, the saturation solubility increased to 13.77 ± 3.50 μg/ml. This was a statistically significant difference between the suspension and nanosuspension.

The dissolution study was performed under sink conditions, obtained by the addition of 5% sodium lauryl sulfate was added to the dissolution medium to increase solubility of folic acid. Within the initial 5 minutes, 78.6% of folic acid dissolved from the nanosuspension and 6.2% dissolved from the suspension. Within 20 minutes, over 90%

of folic acid was dissolved from the nanosuspension. A similar amount of folic acid was dissolved from the suspension under 45 minutes. This difference in dissolution speed was statistically significant (α = 5%).

A stroboscope diode synchronized with the actuation signal of the droplet generator was employed to view the drop formation of the nanosuspension and calculate droplet diameter and velocity. A high degree of accuracy and precision was assessed. No nozzle clogging was observed after printing had been stopped for an hour and no buildup of solid deposits was present in or around the nozzle aperture.

From the data collected by the study, it was determined that formulating a nanosuspension of the poorly soluble model drug folic acid for inkjet printing was advantageous as it increased dissolution speed and had a high level of reproducibility. Good storage stability and low waste were also noted.

4.2 A Step Toward Development of Printable Dosage Forms for Poorly Soluble Drugs. (8)

The objective of this study was formulation of printed dosage forms for a model poorly soluble drug, piroxicam, using piezoelectric inkjet printing and flexography. Piroxicam (PRX) was dissolved in a series of solvents (water, ethanol, 2-propanol, glycerin, polyethylene glycol (PEG), propylene glycol (PG)) and printed onto edible icing paper. The printed dosage forms were characterised using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM−EDX) and the amount of drug was analysed by high-performance liquid chromatography (HPLC). Chemical and physical stability were also tested by HPLC and SEM-EDX. Release profiles were determined by the use of the method suggested by United States Pharmacopoeia for dissolution testing of PRX capsules; simulated gastric fluid media without pepsin.

A Dimatix DMP-2800 printer was used for inkjet printing and a replaceable cartridge with 16 nozzles each producing a typical a drop volume of 10 pL was used. Flexographic printing was performed by a laboratory scale printability tester, model IGT Global Standard Tester 2.

Ink formulated for inkjet printing was produced in differing PEG:ethanol ratios (30:70, 40:60,50:50) and were filtered using a 0.45 and 0.2 µm membrane filters.
Ink formulated for flexography was not filtered and was produced with PEG-400 to produce a solution. 10 µL of the formulas for inkjet printing and flexography were also manually pipetted onto the edible paper. PEG-ethanol and PEG were chosen over other tested solvents such as water, ethanol, 2-propanol, glycerin due to solubility and boiling points. Water was tested as a cosolvent due to an interest in obtaining monohydrate forms of PRX after printing. The inks formulated for inkjet printing were formulated below the saturation point of PRX to prevent aggregation and crystallisation on the nozzle aperture. Viscosity and surface tension were controlled and analysed by the pendant drop method and a rheometer. Viscosity and surface tension was of all PEG-ethanol inks were found to be within the suitable range for inkjet printing. Flexography does not have such narrow criteria for ink suitability. The formation of satellite drops was observed in PEG-ethanol (30:70) ink and when extended printing (overnight) occurred, nozzle clogging was observed for PEG-ethanol (50:50). An ink formulated with the ratio 40:60 avoided these issues and was regarded to be optimal.

Determination of drug amount printed showed a higher relative standard deviation for flexographic printing than for inkjet printing. The theoretical drug amount for inkjet printing was 50.1 µg/cm$_2$ whilst the experimentally achieved amount was 52 µg/cm$_2$. For flexographic printing the actual yield was 7.6 µg/0.5 cm$_2$ if 10 layers were printed but 13.2

µg/0.5 cm$_2$ if 20 layers were printed. This shows a lower drug amount/layer for 20 layers and this was presumed to be a result of a "smearing effect" when printing multiple layers on the same area. Inkjet printing was deemed the more precise method.

SEM analysis showed no drug particles or crystals on the substrate when a solution was used for printing with flexography. Needle-shaped crystals were observed on the substrate surface for the suspension used for inkjet printing. Drug distribution was analysed by SEM–EDX through elemental analysis of sulfur. This method was chosen due to previous difficulties with routine solid state analysis such as X-ray powder diffraction, Raman spectroscopy, near-infrared spectroscopy and attenuated total reflectance spectroscopy. Issues were determined to arise due to the chemical composition of the icing paper substrate. Sulfur was determined to be absent from the substrate, making detection of sulfur by SEM-EDX a method to determine PRX by proxy. The data from SEM-EDX was in agreement with the observed needle crystal structure of the inkjet printed doses. The analysis of the flexographic printed PRX solution found sulfur evenly distributed throughout the edible paper at a low level, although some patterning was also observed. No solid particles in the detectable range of SEM-EDX were indicated from the data.

Dissolution studies showed that printed dosage forms from inkjet printing and flexography showed a drug release of >90% within 5 minutes in simulated gastric fluid. The printed dosage forms were found to be physically and chemically stable for a month under ambient conditions (20◦C–25◦C/30%–40% RH).

The study found piezoelectric inkjet printing to be a more precise and effective method for fabricating doses of the poorly soluble and/or potent drugs. It was also noted that more research was needed for the understanding of drug/substrate interactions, the influence of ink formulation and printing parameters on print quality, the effects of longer term storage and packaging, and the characterisation of printed dosage forms.

4.3 Fabrication of drug-loaded polymer microparticles with arbitrary geometries using a piezoelectric inkjet printing system. (6)

This study explored fabrication of various carrier geometries with an API deposited by a piezoelectric inkjet printer. Paclitaxel-loaded poly(lactic-co-glycolic acid) (PLGA) microparticles were deposited in circles, grids, honeycomb structures and rings and the effect of deposition pattern on drug release behaviour was investigated. The activity of the API was tested on human cervical cancer cells (HeLa line).

The ink was formulated by dissolving PLGA in dimethylacetamide (DMAc))and filtering out insoluble particles; 1% paclitaxel (PTX) w/v was added. Ink formulation was optimised for printing and viscosity controlled by a viscometer. A Dimatix-brand (model not stated) piezoelectric inkjet printer was used and printing occurred in a clean bench. Fluorescein isothiocyanate was added pto the ink to facilitate visualisation of the morphology. A fluorescence microscope and non-contact surface profiler were used to analyse the pattern morphology. Scanning electron microscopy was used to identify morphology changes under release. *In vitro* analysis included utilisation of WST-1-based colorimetric assay to illustrate cytotoxicity and high performance liquid chromatography (HPLC) to analyse the release profile.

The features of the printed geometries are presented in table 2., taken from the study. The printed geometries were considered to be reproducible, precise, and able to be printed at a size amenable to mass production.

Table 2. Comparison of the various geometries printed. Comparisons are made of the drug load and the weight, height, volume and total surface area for the four different geometries. Taken from (5)

Shape	PTX Loading amount (µg)	Weight (µg)	Height (µm)	Volume (µm$_3$)	Total Surface Area (µm$_2$)
Circle	0.34	6.5	28	335728	235123
Grid	0.34	6.5	25	3010000	398800
Honeycomb	0.32	6.0	23	3378033	579740
Ring	0.34	6.5	25	3477864	367405

Phosphate buffered saline containing 0.1% (v/v) Tween-80 was used as a medium to test release rates. This solution was chosen due to poor solubility of PTX in water. A biphasic release profile was observed in all geometries under the study period of 7 days. Fast release on day 1 was followed by continuous release over the following 6 days. The highest rate of release was observed by honeycomb grid geometries where almost all API was released. Approximately 80% of API was released by grid and ring patterns and only 60% of API had been released by the circle shaped microparticles. The Haguchi equation ($Q = kt_{1/2}$) was used to calculate release rate whilst Student's two tailed t-test was used for statistical analysis where statistical significance was accepted at $p < 0.05$.

PTX concentrations of 340 nM to 3400 nM were used in the microparticles for the WST-1 assay and it was shown that whilst a single microparticle loaded with 340 nM PTX reduced HeLa cell viability, the use of 10 PTX-loaded microparticles (3400 nM) reduced cell viability to below 10%.

The study found that manipulation of the printed geometry of inkjet printed API resulted in differing drug release rates, allowing for a high degree of personalisation. The study was successful in showing the viability of an inkjet printed drug delivery system for paclitaxel as cytotoxicity was proved *in vitro*.

4.4 Evaluation of different substrates for inkjet printing of rasagiline mesylate. (9)

Rasagiline mesylate, a low dose antiparkinson agent, was chosen as a model drug in this study which examined the applicability of three different substrates in the production of inkjet printed drug delivery systems. The density of the printed ink was of interest in exploring the viability of flexible dosing through multi-level printing. Characterisation and analysis was performed by polarised light spectroscopy, near-infrared chemical imaging (NIR-CI), SEM, X-ray diffraction, and HPLC.

The ink was formulated by dissolving 100 mg/ml rasagiline mesylate in a solution of 30:70 propylene glycol (PG) and purified water. The viscosity of the ink was tested by use of rheometer and the viscosity was stated to be within the desirable range. The substrates chosen for the study were standard copy paper, impermeable transparency films and orodispersible films. The orodispersible films were manufactured by a solvent-casting method in-house for the study and were composed of 14% hydroxypropyl methylcellulose, 4.7% crospovidone, 4.2% glycerol, and 77.1% water.

An unmodified thermal inkjet printer, Canon Pixma MP495, was used for printing and compatible cartridges (InkJet cartridge 372, print code J3CANOG512) were cleaned

according to an internal procedure. Prior to printing with an ink containing API trial printing runs using a 30:70 PG:water ink to ensure a lack of contamination. Rectangular forms measuring 16 mm × 26 mm were printed using Microsoft Word 2010 and were printed using a single or multiple passes to adjust the amount of layers printed. Printing of multiple levels included a drying time of 60 min at ambient conditions between each print for all substrates but copy paper, which had a higher degree of absorptivity.

The contact angle between ink drop and substrate was measured with the drop shape analysis system Krüss DSA 100 immediately after deposition and was shown to measure 69.0° ± 2.0° on the copy paper, 61.4° ± 1.0° on the transparency film substrate, and 34.4° ± 2.0° on the orodispersible films. The contact angle on orodispersible films was significantly lower than expected and the ink was considered suboptimal for printing on orodispersible films. The use of a different solvent was suggested.

Polarised light microscopy was used to analyse the API-printed substrates. Crystals were not observed on the surface on the copy paper substrate, implying a complete absorption of the ink into the substrate. Crystals were observed on the orodispersible film and transparency film substrates. Crystals were observed on the orodispersible film substrate after two printing passes and the crystals grew with every additional print cycle. Due to the lower contact angle of the ink on orodispersible film substrate, the drops did not coalesce and arranged themselves in a line formation. The crystals visible on the orodispersible film substrate were smaller than those observed on the transparency film substrate - this was ascribed to partial penetration and dissolution into the orodispersible film substrate. The droplets on the transparency film substrate coalesced and remained on the surface of the substrate unchanged, leading to larger crystal formation.

The use of SEM gave data in agreement with the above observations. It was also noted that the rough surface of the copy paper could potentially lead to difficulties identifying crystalline structures. A lack of homogeneity was noted on the surface of both orodispersible and transparency film substrates.

NIR-CI was used to evaluate the dispersion of API on orodispersible and transparency film substrates. It was not performed on copy paper due to the absence of crystals. The data gained from this analysis was also in agreement with previous analysis for doses comprised of multiple printed layers and the similarity increased with increasing layers. For doses comprising of a single layer, the similarity was low as the penetration depth of the NIR light was higher than the thickness of the printed spots.

X-ray diffraction was used to analyse crystallinity and found no peaks characteristic of crystal structure of the API due to a near complete absorption of into into substrate. Partial recrystallisation was observed on orodispersible and transparency film substrates.

When API content of the printed doses was analysed, it was found to increase with repeated printing cycles and the best linear correlation was observed for copy paper. The standard deviation noted for orodispersible films was considered unacceptable and the substrate featured the lowest transferred amount for all substrates. This was in part attributed to shear forces and a thicker printing substrate resulting from an intermediate liner being used when printing orodispersible films. This was stated to result in a smearing effect. 8-9 printing cycles were determined to be necessary for printing a therapeutically relevant dose of the API. During HPLC analysis, peaks related to degradation of the API were observed for printed transparency film and copy paper substrates.

The experimental study found that several issues presented when using a thermal inkjet printer to produce therapeutically relevant doses of rasagiline mesylate onto a variety of substrates. It was however noted that it was possible to successfully print a range of doses onto copy paper, indicating the promise of edible paper as a substrate for printing low dose drugs.

4.5 Behavior of printable formulations of loperamide and caffeine on different substrates—Effect of print density in inkjet printing. (10)

The effect of substrate and inkjet print density of the model drugs loperamide hydrochloride and caffeine was investigated in this study. Edible icing sheet and an in-house produced cellulose film were the substrates tested. Analysis was performed by energy-dispersive X-ray analysis (SEM-EDX) differential scanning calorimetry (DSC), HPLC, and SEM.

An ink containing caffeine was prepared by dissolving 20 mg/ml of API in a solution of propylene glycol (PG) and purified water at a ratio of 30:70. An ink containing loperamide was formulated with ethanol as a solvent, with 50 mg/ml being dissolved in a 40:60 PG/ethanol solution. Both inks were filtered twice with membrane filters of 0.45 μm and 0.2 μm. Viscosity was tested in triplicate using a rheometer and surface tension of the base inks was tested using the pendant drop method.

The cellulose films were produced in-house and were composed of 5% hydroxypropylcellulose (HPC). Polyethylene terephthalate (PET) films were used as a reference point.
Printing was performed by a Dimatix DMP-2800 piezoelectric inkjet printer using a replaceable cartridge with 16 nozzles producing a typical drop volume of 10 pl. Variation in printed dose was achieved by varying the drop spacing of jetted ink between 10 and 50 μm whilst maintaining a standard print area of 4 cm$_2$ or by varying the print area between 0,4 cm$_2$ and 4 cm$_2$ whilst maintaining a standard and unchanged drop spacing of 10 μm.

Contact angles of the inks on the substrates was measured by a CAM 200 contact angle goniometer. It was found that the contact angle for loperamide ink was lower for all substrates in comparison to caffeine ink. Similarities between the behaviour of the two inks was noted on PET films and HPC films where droplets were observable after 3 min. This is in contrast with the behaviour of both inks on icing sheet substrate where the contact angle decreased rapidly as the droplet was absorbed by the substrate after 1 s.

SEM analysis was used to determine the morphological features of the printed API. It was observed that loperamide ink printed onto PET films solidified into smooth-surfaced droplets, which were visible for both drop spacings. On the relatively smoother HPC film, the drop formation was only visible at high drop spacings which led to the conclusion that the loperamide ink was dissolved fully into the substrate when a lower drop spacing was used and partially so at a higher drop spacing. No droplets of loperamide ink were observed on the surface of the icing sheet substrate. This contrasts with the behaviour of the caffeine ink where crystallisation was observed on all substrates. The size of the crystals depended upon the dose and the absorbance of the substrate, with the largest crystals forming on the PET film at the higher dose. A lack of homogeneity was observed as high and low intensity areas of crystals occurred. This was attributed to the ink coalescing before recrystallisation occurred. PET film substrate showed smaller needle formation at low dose and a higher degree of homogeneity. Crystals were observed as protruding from the HPC film which was attributed to partial absorption. Caffeine needles were also observed on the icing sheet substrate surface although they were the smallest observed on all substrates. SEM-EDX analysis was performed on substrates with loperamide ink to determine the distribution of chlorine, an element only present in the loperamide molecule and absent from all substrates. Chlorine was detected on the surface of the PET substrates and high dose HPC substrate. It was not observed on the surface of low dose HPC substrate or on either dose printed onto icing sheet substrate. It was detected at noise levels for icing sheet substrates, indicating absorption into the substrate.

X-ray diffraction was performed to confirm the crystallinity of both APIs printed onto the substrates. Both APIs were crystalline materials in their pure form but no characteristic crystalline peaks of loperamide were observed for any substrates. It was presumed that loperamide took an amorphous form or a molecular dispersion after ink deposition occurred. Characteristic crystalline peaks of caffeine were observed for caffeine ink printed on all substrates and it was determined that the caffeine recrystallised as stable form II with potential inclusion of a hydrate form. The caffeine powder used for ink formulation was determined by X-ray diffraction to be the same stable anhydrous form. DSC studies supported the results of X-ray diffraction.

The theoretical amount of API in the printed dose with a set area was determined to increase as a function of drop spacing decreases. This was observed for the printed forms of loperamide and caffeine although the actual amount of drug determined for both APIs was more than the theoretical amount but the standard deviation for the results was very low. It was noted that more rigorous determination of droplet size/volume was necessary to better calculate theoretical API amount in the finished product.

The suggested that the use of inkjet printing was suitable for flexible doses of caffeine and loperamide and that the use of substrate can allow for increased variation and control of the crystalline behaviour of the API.

4.6 Inkjet printing of drug substances and use of porous substrates towards individualised dosing. (11)

Inkjet printing was used to print doses of the model drugs theophylline, caffeine, and paracetamol onto various porous substrates and the penetration and crystallisation behaviour of the drugs was studied. Analysis was performed by liquid chromatography–mass spectrometry, light microscopy and scanning probe microscopy (SPM) measurements, time-of-flight secondary ion mass spectroscopy (ToF-SIMS).

Ink solutions of the three model drugs were created by dissolving the drug in a 30/70 propylene glycol and purified water solution which was filtered through a 0.2 μm polypropylene membrane filter. The concentrations of the drugs in the solutions were 9.9 mg mL−1 for paracetamol, 5.8 mg mL−1 for theophylline, and 19.3 mg mL−1 for caffeine. Viscosity and surface tension was controlled and measured before printing.

Printing was performed by a Dimatix DMP-2800 inkjet printer and the substrates used were uncoated paper, coated paper, and polyethylene terephthalate (PET) film. As a reference, freshly cleaved mica was used for scanning probe microscopy (SPM).

SPM was used in the characterisation of the three substrates. The three different substrates were chosen to represent various levels of porosity and permeability; pigment-coated paper being a porous substrate with low permeability, uncoated copy paper as a porous substrate with high permeability, and PET being a non-porous substrate. The root mean square was, in addition to topography data gained from SPM, used to ascertain roughness and surface porosity. Pigment-coated paper had a root mean square of 90 ± 5 nm and PET had 33 ± 2 nm. Height profiles in addition to a root mean square of over 2 μm show the dramatically higher degree of roughness and porosity of uncoated paper; attributed to the 3D network of fibre structure.

Evaluation of the printed drug solutions were performed with light microscopy, SPM, and ToF-SIMS. Light microscopy showed crystalline behaviour on the PET substrate and penetration of the more porous paper substrates. Caffeine showed a partial crystallisation on the coated-paper substrate. SPM allowed for further investigation into the penetration and crystallisation habits of the different drug solutions on the substrates which supported the findings by light microscopy. The ToF-SIMS analysis allowed for

identification of drug and drug compounds on the surface of the substrates. The data peaks for the drugs showed no overlap with those of the substrates and this information was used to map the printed drug on the substrate. The strongest signal was detected on the PET substrate, which was in agreement with the non-absorbing nature of the substrate. The data also showed an almost complete absorption of drug into substrate for the uncoated paper had occurred. Some drug was observed to have remained on the surface of the coated paper substrate.

Analysis of drug content showed a lack of agreement between theoretical and actual yields. The standard deviation for all samples was circa ± 4%. The printed yield of caffeine was 29% higher than the theoretical yield whilst the printed yield for paracetamol was 31% lower than the theoretical. Theophylline had a printed yield of 25% higher than the theoretical. It should be noted that no degradation of substances was observed.

4.7 Inkjet printing of proteins: an experimental approach. (12)

Proteins offer a promising target for pharmaceutical development but current delivery methods present a multitude of issues related to retention of structure and bioavailability post administration. This experimental study explored the practicalities of using thermal inkjet printing as a means of printing lysozyme and ribonuclease-A onto polyethylene terephthalate sheets to create printed films. The research was also focused on buccal delivery. Analysis was performed by protein assay kit, enzymatic activity testing, circular dichroism spectroscopy (CD), and statistical analysis.

Statistical analysis was conducted with Minitab Release 17 ® software and Student's *t* test was used in addition to Tukey's *post-hoc* comparison and one way ANOVAs were used for multiple comparisons. Results were expressed as an average and standard deviation of three replicates and a $p < 0.05$ was considered statistically significant.

The printer used in this experiment was a Hewlett Packard Deskjet 1000 adapted for the biologics printing process. The cartridges used were printer-compatible Hewlett Packard cartridges which had been emptied of ink, cleaned with hydro-alcoholic solution and undergone 3 sonication cycles.

The proteins used were lysozyme from chicken egg white (14.3 kDa) and bovine pancreatic ribonuclease (13.7 kDa); sodium deoxycholate was added to the ink formulation as a permeation enhancer. Inks without the inclusion of sodium deoxycholate were also formulated. Sodium deoxycholate is conventionally added to buccal delivery methods to increase permeation. The ink was formulated with purified water as both proteins have high solubility in water. Previous research had shown that a 70:30 v/v solution of protein solution and glycerol was suitable for printing as the viscosity is similar to the traditional ink used. The inks were printed in concentrations of 0.15, 0.5, 3.5, and 10 mg/ml in a series of 4, 9, 16 and 49 cm$_2$.

Printing efficiency was evaluated at ink concentrations of 3.5 and 10 mg/ml printed in triplicate at a size of 49 cm^2.

Protein quantification was performed for both lysozyme and ribonuclease-A with a Micro BCA protein assay kit with the reagent ratio of 25:24:1 for reagents A, B, C. A constant volume of reagent and printed sample were used in the microplate assays and the printed sample measurements were performed in triplicate.

The enzymatic activity retained after printing was evaluated by different methods for the two proteins. A modified version of Shugar's method was used for lysozyme, wherein the lytic activity of lysozyme was tested over *M. lysodeikticus* cell walls. The decrease in absorbance at 450 nm was recorded at 1 s intervals for 5 min using a Lambda 25 UV/Vis spectrometer. This was tested against the reagent blank with only substrate and water and the measurements were recorded in triplicate. A modified method developed by Crook was used to test enzymatic activity of printed ribonuclease-A and involved testing of the ability of the ribonuclease to hydrolyse cytidine 2, 3-phosphate to cytidine 3-phosphate. The activity was observed by measuring the increase of absorbance at 284 nm using a Lambda 25 UV/Vis spectrometer. This was also tested against the reagent blank with only substrate and water and the measurements were recorded in triplicate. The results showed a tendency of lower enzymatic effect at lower ink concentrations and a similar trend was observed in regard to printed area. At concentrations 0.15, 0.5 mg/ml showed an effect of 30% in comparison to concentrations 3.5, 10 mg/ml where an effect of 80% was observed. No significant difference was observed between concentrations 0.15, 0.5 mg/ml or for concentrations 3.5, 10 mg/ml. No differences were observed regarding the inclusion of sodium deoxycholate for lysozyme ink at low concentrations but a slight but statistically significant lowering of enzymatic activity was noted for the higher concentration inks. A similar but less pronounced trend was observed for ribonuclease-A inks.

The CD analysis was conducted in a series of three consecutive measurements and the average spectra was recorded. The results were compared to standard solutions of the protein. The results showed no major differences in spectra for printed inks (both including and excluding sodium deoxycholate) when compared to spectra of standard solutions of lysozyme.

The study concluded that thermal ink jet printing was an efficient and practical method of producing films of biologics without compromising protein activity.

4.8 Inkjet printing as a novel medicine formulation technique. (13)

The suitability of inkjet printing a controlled release formulation of the poorly soluble drug felodipine was explored in this study. Several controlled release formulas based on polyvinyl pyrrolidone (PVP) and felodipine were deposited by a piezoelectric printer onto a hydrophobic substrate. The ink was deposited as microdots - droplets with a sub 100 µm diameter and later characterised using atomic force microscopy (AFM), scanning thermal microscopy, nanothermal analysis, attenuated total internal reflection infrared spectroscopy, and Confocal Raman microscopy.

The various inks featured different ratios of felodipine to PVP at the following ratios: 1:10; 1:5; 1:2; 1:1; 2:1 w/w. Felodipine and PVP is a was considered a well established and researched system and published bulk methodologies were used to validate the efficiency of inkjet printing. As felodipine is soluble in ethanol, ethanol was used as a solvent and dimethyl sulfoxide was used as an additional solvent and to increase the boiling point of the solution. This was necessary to prevent premature evaporation of the ink leading to nozzle blockage.

A Gesim A010-201 PicPip piezoelectric printer was used and features a nozzle orifice of 90 µm and a standard drop size of 300 pL. A standard drop of a 1:1 felodipine/PVP ink was stated to contain 0,3 µg felodipine and thus a dose of 2,5 g felodipine requires 9000 microdots. Therefore, this study included depositions created by single and multiple drops.

AFM showed no crystallisation behaviour by felodipine/PVP ink at a ratio of 1:10 in depositions up to 40 drops. This is consistent with the crystallisation behaviour of pure felodipine. Crystallisation was observed with extended spraying (> 1 minute) of the same spot.

Localised thermal analysis showed that the thermomechanical properties are a contributory factor in the experimental measurements or the sample did not ideally match the assumptions of the felodipine and PVP model.

Analysis by infrared spectroscopy was used to consider the intermolecular interactions between felodipine and PVP, specifically the formation of hydrogen bonds between an amine group of felodipine and carbonyl groups of PVP and other felodipine molecules. Interactions were observed in inks with ratios 2:1; 1:1; 1:2 and was reported as consistent with similar bulk formulations and suggestive of an amorphous rather than crystalline character of felodipine. However, interactions were observed at a higher ratio of felodipine than previously shown in the literature. This was theorised to be the result of a smaller amount of product being used in inkjet printing as opposed to bulk solid dispersion methods.

Confocal Raman microscopy was used to analyse the homogeneity of the depositions, which were found to be largely homogeneous - lower concentrations were observed at the edges of depositions. The dissolution rate was examined by the construction of a calibration curve based on the Raman spectra with increasing dissolution time in deionised water. PVP was shown to be preferentially removed and high loadings of felodipine had a poor dissolution rate under the studied timeframe of 10 s.

The study determined that the poorly soluble drug felodipine was able to be successfully inkjet printed and characterised. Release rates were considered favourable due to the addition on PVP. The technology was deemed suitable for scaling up and the microdot array was considered suitable for future applications for multidrug administration.

4.9 Inkjet printing of insulin microneedles for transdermal delivery. (14)

Metal microneedles were coated with experimental ink formulations composed of bovine insulin and a polymer in this study. Gelatin, poly(2-ethyl-2-oxazoline) (POX), trehalose and polyvinyl caprolactame-polyvinyl acetate-polyethylene glycol (SOL) were used to formulate inks. Piezoelectric inkjet printing was used to apply the insulin ink in layers. The various polymer-insulin systems were analysed and evaluated as to their suitability for printing and as for drug delivery. The analysis methods used were atomic force microscopy (AFM), circular dichroism (CD), HPLC and SEM. The release rates were assessed by the use of Franz diffusion cells from microneedles applied to porcine skin.

The inks were formulated with deionised water and a polymer/insulin ratio of 1:2. To assess the insulin-coated amount, three microneedle assays were each separately dissolved in a phosphate buffer and the amount of insulin was determined by high-performance liquid chromatography (HPLC).

A PicPip 300 was the piezoelectric dispenser used and the pipette/nozzle type was stated as GESIM Nanoplotter II. The insulin/polymer formula was deposited in a series of jetting cycles so as to build layers. 50 jetting cycles were used for each ink formula. A stroboscope was employed to ensure correct drop formation. Due to the small volume of the drops and the preciseness needed, the microneedles were positioned at 45° relative to the dispenser. By rotating the microneedle array, both sides of each individual microneedle was able to be coated in an even manner.

AFM analysis was used to determine homogeneity and roughness of printed polymers and printed insulin/polymer formulations. It was shown that the inclusion of a high level of

insulin increased the roughness of all formulas except POX. A low level of roughness was stated as desirable due to a lowered risk of the coating adhering to the skin rather than being transported transdermally during microneedle piercing. However, the increase in roughness was not considered to be significant or a risk as the surfaces were deemed relatively smooth. The deposition was determined to be uniform and homogenous and without phase separation. This was noted as necessary for a fast release of insulin.

Analysis by CD spectrometry was performed to evaluate any denaturation and helix coil transformation of insulin as a measure of the suitability of insulin to be printed and subsequent stability in solid state. Insulin absorption bands associated with alpha helices and beta sheets were observed in the experimental spectra. Due to this agreement, good stability of insulin within the polymer was assumed. The experimental spectra of insulin/SOL ink and insulin/trehalose ink matched the absorption bands to a higher degree than the other inks, implying a higher degree of stability in those formulations. A lower degree of stability was assumed for insulin/POX ink due to a mismatch at the second peak - implying negative processing conditions or strong molecular interactions between polymer and insulin.

Release of insulin from microneedles coated with polymer/insulin formulations was assessed by the use of Franz cell diffusion and porcine skin. The insulin/SOL formulation released circa 65% of insulin load within the first ten minutes whilst insulin/gelatin released a similar amount. Insulin/trehalose released circa 40% and insulin/POX only released circa 30%. After a duration of 30 minutes, SOL had released circa 95%, gelatin released circa 85%, trehalose released circa 75% and POX released circa 40% of insulin load. The experiment was allowed to run for 60 minutes and SOL was the only polymer that released 100% of the insulin load, reached at 40 minutes. It was concluded that only gelatin and SOL allowed for a release rate suitable for delivery into the bloodstream.

Microneedles coated with an ink of insulin and SOL were considered successful under the parameters of this study. It was concluded that it was possible through the use of inkjet printing to print layers of a stable and homogeneous ink containing insulin whilst retaining secondary structures such as alpha helices and β–sheets intact.

4.10 Inkjet printing of transdermal microneedles for the delivery of anticancer agents. (15)

The coating of stainless steel microneedles with anticancer agents by piezoelectric inkjet printing was explored in this study. 5-Fluorouracil, curcumin and cisplatin and the hydrophilic graft copolymer Soluplus® (polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol) were formulated in different ratios to create inks that were suitable for printing. HPLC and atomic absorption spectroscopic analysis were used for drug quantification. Release rates of the coated microneedles were tested *in vitro* by Franz diffusion cells with dermatomed porcine skin.

The microneedles were crafted using an infrared laser with the designs having been drafted in CAD files. After cutting, the microneedles were manually bent post washing. Electropolishing occurred in a bath containing a 6:3:1 mixture of glycerin, phosphoric acid, and water; this reduced the thickness of the microneedles to 50 μm.

Inks were formulated by dissolving polymers in deionised water or ethanol and subsequently adding the API or the control model substance, sodium fluorescein. Inks of various compositions were formulated with 3% API or sodium fluorescein and 3, 6 or 9% polymer. Soluplus® was chosen as a suitable polymer due to it increasing the water solubility of the API, a desirable trait given the insolubility of cisplatin and curcumin.

A PicPip 300 piezoelectric dispenser was used in combination with a GESIM Nanoplotter II pipette/nozzle. The droplets formed were in the range of 300 pL in volume and the microneedles were positioned at 45° relative to the dispenser due to the small volume of the drops and the preciseness needed. A stroboscope was employed to ensure correct drop formation and drop dispension. A coating cycle consisted of the positioning of 4 drops longitudinally to the axis of each microneedle and the coating cycle was repeated 1, 2, or 5 times. The microneedle array was also rotated so that both sides of each individual microneedle was coated in an even manner.

Abdominal porcine skin was dermatomed to a thickness of 750 and 900 μm, cut into discs and placed in a phosphate buffer solution for 2 h and removed and kept at room temperature for 15 min prior to use for the Franz cell diffusion tests.

UV scanning and SEM showed precise coating of microneedles without coating other regions of the metal microneedle substrate. This was observed for all inks with varying polymer ratios and sodium fluorescein was shown to be deposited homogeneously and without any void areas or clumps of the model substance. Multiple thin layers were able to be printed by all ink formulas, which avoided the microneedle tip becoming bulky. A smooth surface was also achieved. The API dosage was determined by testing human skin epidermoid carcinoma cell viability at various doses. This testing concluded that the APIs had differing potencies and so different concentrations of API were used in the inks.

The dissolution rate of the APIs and control was studied by the use of Franz diffusion cells wherein the coated microneedle was inserted into porcine skin and the release of the API or control diffused into the chambers and travelled to the receptor compartment. An increase of polymer in the ink resulted in a slower or controlled release rate for all APIs and control. The release rates of the control and 5-fluorouracil were reported as similar. The control showed a rapid release where 1:1 and 1:2 sodium fluorescein/polymer inks released 65% and 45% respectively after 1 h. The release rate of 5-fluorouracil was somewhat slower, possibly due to the drug being more hydrophobic than the control. A significantly slower release rate was observed when the thicker (900 μm) porcine skin was used with circa 35% of the drug load reaching the receptor compartment after 1 h.
All curcumin/polymer inks showed a rapid release and 56 - 95% of drug load being released after 1 h. The variation in released percentage is a result of a higher amount of polymer and thicker skin leading to lower percentages. A 1:2 curcumin/polymer ratio was considered ideal as it released 96% drug load after 1 h. It was concluded that the addition of Soluplus® polymer increased the solubility (and thus bioavailability) as a lower curcumin/polymer ratio led to a lower total release. Testing with thicker (900 μm) porcine skin led to slower release rates, a smaller amount of API reaching the receptor chamber and a higher drug load remaining in skin tissue.
Cisplatin release was also rapid, despite the poor solubility of the drug. API/polymer ratios of 1:1 and 1:2 presented with similar release profiles of 82% and 77% of drug load reaching the receptor chamber after 1 h. An increase of polymer in the ink resulted in a delayed API release - only 57% reached the receptor chamber after an hour. Thicker porcine skin resulted in a poor release profile.

The study found that piezoelectric inkjet printing was a suitable method of coating microneedles that resulted in a uniform and reproducible coating. The optimised ink formulation and use of Soluplus® resulted in rapid release rates and improved solubility.

Table 3. Table compiling main focus, results, and analysis methods used for the chosen studies.

Study	Main Focus	Result	Analysis Methods
(7)	Poorly soluble drugs	Successful formulation of folic acid into a nanosuspension and subsequent inkjet printing	LD, PCS, X-Ray Diffraction, HPLC, dissolution study
(8)	Poorly soluble drugs	Successful printing of piroxicam using inks with various solvents using flexography and piezoelectric printer, showing IJP as a superior method	SEM-EDX, HPLC, SEM, dissolution study
(6)	Technical parameters	Successful inkjet printing of paclitaxel in various geometric patterns for manipulation of drug release	SEM, fluorescence microscopy, HPLC, WST-1 colorimetric assay
(9)	Technical parameters	Partially successful inkjet printing of rasagiline onto various substrates	NIR-CI, SEM, HPLC, X-Ray diffraction, Polarised Light microscopy
(10)	Technical parameters	Successful inkjet printing of loperamide, caffeine onto various substrates	SEM-EDX, DCS, HPLC, SEM, X-Ray diffraction,
(11)	Technical parameters	Successful inkjet printing of caffeine, theophylline onto substrates with varying porosity	ToF-SIMS, SPM, light microscopy, liquid chromatography-mass spectrometry
(12)	Printing of proteins	Successful inkjet printing of ribonuclease-A and lysozyme as a film	CD, protein assay, enzymatic activity testing
(13)	Poorly soluble drugs	Successful use of inkjet printing in manufacture of controlled-release doses of felodipine	AFM, scanning thermal microscopy, nanothermal analysis, confocal Raman microscopy, attenuated total internal reflection infrared spectroscopy
(14)	Microneedles; printing of proteins	Successful inkjet printing of insulin onto microneedles	AFM, CD, HPLC, SEM, Franz diffusion cells
(15)	Microneedles; poorly soluble drugs	Successful inkjet printing of anticancer agents onto microneedles	SEM, HPLC, atomic absorption spectrometry, Franz diffusion cells

5. Discussion

Inkjet printing shows great promise in drug discovery, formulation and administration due to the technology allowing for a high level of flexibility and efficiency. The application of IJP within drug production may lower wastage costs, allow for previously discarded APIs to be further explored, increase compliance and accessibility of drugs (1, 3). Standardisation of equipment is required in addition to ensuring the safety of ancillary equipment. Similarly, formulation of carrier fluids for the API requires further development so that it fulfills both the physical and legal criteria for a successful product. As the physical property varies with the API, research into suitable "base" formulas for classes of API would benefit large-scale production (1, 5, 10). The suitability of IJP as a tool for drug personalisation is huge as the technology allows for a high degree of dose control and release by manipulation of API deposition pattern in addition to personalised multi-drug formulations. Medical devices such as microneedles may also benefit from IJP technology as a means to reduce production costs whilst increasing yield. However, it is not without drawbacks, which will also be discussed in this review. This review centres on a limited number of studies to explore the practicalities and viability of inkjet printing and to answer the questions posed. A literature study was considered optimal in answering the questions posed as the study type allows for a relatively large amount of information from experimental studies from all countries to be accessed, given that they conform to the search criteria. Studies were largely from the U.S.A or Europe. The scope of the posed questions and the novel nature of the technology within the pharmaceutical sector meant that reviewing selected experimental studies allowed for a good examination of current developments and potential applications. This allowed for a solid foundation on which to find answers to the posed questions. The time frame allowed for this study also made a literature study an ideal choice. A limited number of relevant studies led to difficulties in forming fully informed and entirely nuanced answers and the fact that certain researchers were involved in more than one of the selected studies opens up issues with bias and methodology. The latter issue is unavoidable given the relatively small amount of studies fitting the method criteria and being relevant to the questions posed but is still noteworthy. Whilst it appeared that APIs were provided by pharmaceutical companies in certain studies, it is not obvious that there is a bias as a result. No studies selected were funded by pharmaceutical companies. For a clearer result, the questions posed could have been made more narrow. Doing so would have resulted in a more myopic view of the technology and its capabilities rather than giving wider focus to potential applications and benefits to patients and the pharmaceutical industry. This was deemed more fitting for a literature study exploring the possibilities of a novel technology.

The studies chosen for this review largely focus on three areas (i) printing of traditionally challenging APIs; (ii) the use of inkjet technology for coating microneedles; (iii) technical parameters required for successful inkjet printing. Despite covering different topics, many of the studies use the same analysis techniques, allowing for better comparison and evaluation of studies. Two studies used thermal inkjet printers whilst eight used piezoelectric printers (4 Dimatix models, 3 PicPip models), making direct comparison of results difficult. However, overarching similarities occur and the studies are therefore included as the results are deemed to be relevant.

5.1 Poorly soluble APIs and Proteins
Although proteins and APIs with poor aqueous solubility were included in many of the chosen studies, they were more focal in the investigation of studies (7), (12), and (15). Collectively, the studies indicate that in combination with a well-formulated ink, inkjet printing can allow for higher solubility and bioavailability of poorly soluble or protein APIs. Study (7) evaluated the effectiveness of formulating poorly soluble drugs into a suspension and nanosuspension as an ink. Analysis by LD and PCS was undertaken to ensure a homogeneous particle size, an important factor for printing consideration and dose characterisation. Measurements were performed in triplicate, offering a high level of

reproducibility and accuracy given the low standard deviations given. The study evaluated the physical and chemical stability of the printed forms under simulated storage and freight conditions. After a 30 day period, the results showed promising results for both chemical and physical stability, necessary aspects for the commercialisation and scaling up of inkjet technology. Even if doses are produced and administered on or near site, the stability of the ink is of importance as it is not practical or feasible for on-site ink production (7).

Study (12) focused on the viability of printing protein-based APIs. The studied delivery method was buccal films, chosen as an alternative to injection - a common delivery method for biologics. Similar to study (7), measurements were taken in triplicate and statistical analysis was performed to give statistically significant and reproducible results. The enzymatic activity of the two proteins was compared against controls as a method of proving the viability of protein printing; analysis of structure was performed by CD as a secondary testing of structure. Both methods were in agreement. As secondary structure is inherently tied to activity the analysis methods confirm each other. It was found that at higher ink concentrations with the inclusion of deoxycholate showed lower enzymatic activity after printing for both proteins - attributed to the thermal inkjet process leading to denaturation. This could be avoided by the use of non-thermal inkjet methods such as piezoelectric ink jets, although that would confer a relatively higher price point for production. No dissolution testing occurred which led to an incomplete view of the viability of the produced dosage forms, despite the study showing proof of principle (12).

Studies (8), and (13) included model drugs with low aqueous solubility and examined the factors relating to ink formulation and release profile.
Study (8) compared the effectivity of inkjet printing in comparison to flexography as a method of printing oral delivery systems. Several solvents were explored but only one for each technology was deemed suitable. Differing ink formulations were considered and an optimal PEG:ethanol ratio was found for inkjet printing piroxicam – it was noted that the requirement of tailoring an ink formula for a specific API was one of the drawbacks of inkjet technology. The formula was also shown to successfully print precise and reproducible oral dosage forms with a homogeneous API distribution. Issues were encountered in drug characterisation by standard solid state analysis methods, leading to SEM-EDX analysis to be used. This exemplifies the concerns related to dosing accuracy and quantification on a commercial scale where SEM-EDX may not be suitable or possible. Dissolution testing showed an increased rate of dissolution for both printed forms (8).

Ink formulation was a prime concern in study (13), which examined the success of inks with various felodipine:polymer ratios. Concentration of API in ink and subsequent release profile were considered as they are important factors in personalising medicine. Standard solid state analysis was performed on the printed doses in addition to thermal analysis for evaluation of homogeneity, dispersion, and felodipine-PVP interactions. Thermal analysis presented information that differed from the theoretical but was still consistent with a homogeneous dispersion of felodipine. This was supported by data from solid state analysis methods. Dissolution data showed difficulties when higher doses of drug load were used, an issue of significance regarding flexibility of dosing and personalisation of dose. It was however noted that higher doses could be achieved by a larger amount of droplets printed rather than altering the API ink concentration. This would likely entail a drawn-out printing process as it was found that crystallisation occurred during lengthy (> 1 min) printing of the same spot. This was despite PVP being added to the ink formula as a means of reducing crystal formation; the amorphous form was desired due to increased solubility. The use of statistical analysis was not mentioned, nor was the amount of measurements taken from each sample leading to a lower degree of accuracy in the results (13).

5.2 Microneedle Coatings

Studies (14) and (15) explore microneedle technology in combination with inkjet printing. The studies also utilise either poorly soluble drugs (14) or proteins (15) in conjunction to microneedle technology.

The suitability of microneedles in administering insulin was explored in study (14). Four polymer ink bases were evaluated in regards to insulin structure retention, quality of printed layers and dissolution. Drug characterisation did not occur outside of SEM evaluation and dissolution testing but viability of insulin to be printed was assessed by CD which found secondary structures intact for 2 out of 4 polymer bases. Smoothness of printed surface was another criteria studied and AFM found that a higher concentration of insulin led to decreased smoothness. Porcine skin and Franz diffusion cells were used to test release rates, a factor of high importance for insulin. Rapid release rates were shown by 2 out of 4 polymers, with SOL being the only polymer with suitable release rates and retained insulin structure. This highlights the importance of ink base due to negative interactions between ink and API. The study does not provide information on statistical analysis or if measurements were taken in multiples, which calls into question the reproducibility - despite the successful production of insulin coated microneedles (14).

Study (15) evaluated the use of poorly soluble drugs 5-fluorouracil, curcumin and cisplatin as coatings for microneedles. The APIs used are commonly administered by infusion as part of a chemotherapy regime and so the successful formulation on coated microneedles could improve the quality of life of the patient during treatment. Several polymeric layers of inks containing differing concentrations of API were successfully printed with a high degree of precision and reproducibility. API content of printed ink was not characterised in this study, a necessity for commercial production and especially relevant when potent APIs are explored. A control substance, sodium fluorescein, was however used to evaluate the homogeneity of distribution in the printed coating, allowing for some degree of characterisation. API dissolution was tested using a Franz cell diffuser showed that the inclusion of the polymer Soluplus - chosen due to solubilising properties as a result of its amphiphilic nature - did increase solubility in the printed microneedle coatings. Issues with dissolution rates were observed when 900 µm porcine skin was used, an issue that is potentially overcome by modifying microneedle length (15).

5.3 Technical Parameters

As inkjet printing is not an established technology and does not have a large body of literature or industry standards, it is of importance to evaluate and test the technical parameters of ink formulation, substrate composition, deposition patterns amongst other factors. All selected studies therefore include at least one of the aforementioned factors as an aspect which is evaluated and tested but studies (6), (9), (10), (11), and (14) placed more weight on the study of the necessary parameters for successful printing. Deposition geometry was the focus of study 3, in which an ink formulated with the API paclitxel and fluorescein 5-isothiocyanate was printed in a variety of patterns to analyse release profiles. The printed geometries included circles, grids, honeycomb structures and rings which were subjected to analysis by fluorescence microscopy, SEM, HPLC, and WST-1-based colorimetric assays. Statistical analysis was also performed and measurements carried out in triplicate, ensuring a high degree of replication. The release profile for all printed geometries was biphasic and dependent on surface area. Cytotoxic effect was shown by the microparticles and SEM showed a breakdown of structure over the course of 6 days. The stability of the microparticles is dependent of its geometry but ink formulation is also likely to affect the stability. This shows that, in the case of the ink studied, it is most suitable for on-site printing and administration (6). The study also illustrates the convenience of the addition of fluorescent components to the ink as a means of controlling deposition and composition, an aspect reflected in study (15).

Study (9) was the second study to use a thermal inkjet printer rather than a piezoelectric inkjet printer. The reason for this choice was not clear although thermal inkjet printers are more widely accessible. The study analysed the printed material through polarised light spectroscopy, near-infrared chemical imaging (NIR-CI), SEM, X-ray diffraction, and HPLC. Statistical analysis was also utilised. The printed material was characterised and the crystal structure or lack thereof was determined, allowing for the evaluation of three substrates for oral dose delivery. Issues related to thermal inkjet printing related to it not being a "no-contact" technology were present. Should the study be repeated with the use of a piezoelectric inkjet printer, some issues would likely be resolved. Issues regarding larger doses would however likely still present. The study presented the future development of orodispersible films with a higher degree of absorbance and mechanical strength as a promising substrate but stated that copy paper was currently a superior substrate. This is similar to the findings of other studies which find porous and fibrous substrates to allow for formation of amorphous API forms and good absorption of ink allowing for better dose flexibility (9).

Loperamide and caffeine were used in study (10) to investigate the effect of dose adjustment based on adjusting drop spacing and print area on two substrates. Analysis was performed by SEM-EDX, differential scanning calorimetry, HPLC, and SEM. Adjustment of drop spacing was found to be a superior method of dose adjustment compared to adjustment of printed area as it allowed for better control over dose and crystalline behaviour. This study also showed that substrates with a higher degree of absorbance allow for amorphous drug forms and thus a better dissolution rate. A difference between theoretical dose and printed dose was observed although the standard deviation was not considered to be significant (10).

Study (11) examined three substrates of varying degrees of porosity in the printing of inks containing paracetamol, caffeine, or theophylline. The study demonstrated the extreme degree of flexibility present in terms of substrates for oral delivery. Similar to other studies, porosity was deemed a desirable trait due to allowing a higher degree of homogeneity, penetration of API and control of crystalline structure. A significant difference between printed dose and theoretical dose was noted. As this is a recurring issue noted in several studies, the suitability of current means of drop volume determination and dose prediction based on those results is lacking and other methods must be taken into consideration. The study showed a good degree of reproducibility and drug characterisation through solid state analysis. The degradation of APIs during the study period was also investigated and showed good stability of the printed doses. The longer term stability of printed doses is an important aspect that has not been considered in the chosen studies, likely due to the relative youth of the technology within the pharmaceutical field. It is an aspect that requires further study should inkjet printing be considered on a commercial scale (11).
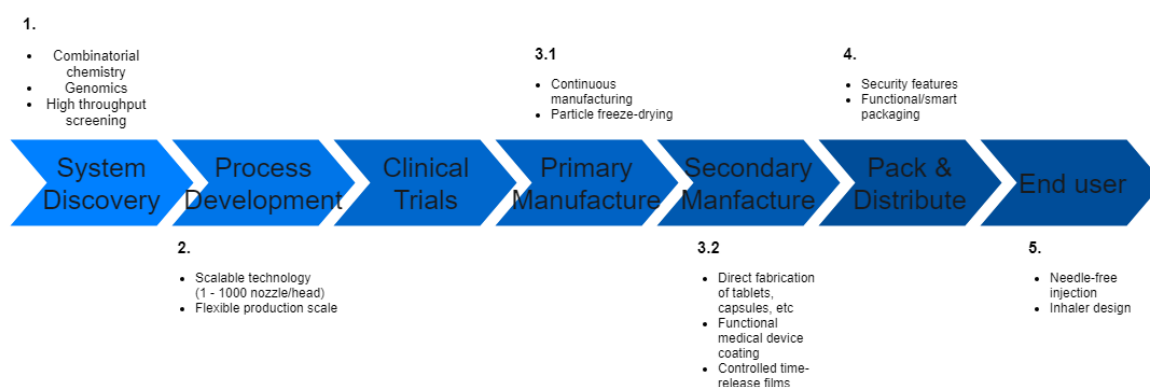

Figure 3. A simplified manufacturing innovation pipeline illustrating points where inkjet printing can be utilised. Taken from (2)

5.4 Economic factors

One of the strengths of inkjet printing from a purely economic standpoint is the flexibility and wide range of application within the production chain, as can be seen in fig. 3. Due to novel technologies and treatments arising, the drug supply chain and manufacture needs to adapt if current profit margins are to be sustained. The "blockbuster" business model of drug development and production/manufacture, where a product is developed and achieves a peak sale of over 10 billion SEK, has been criticised in recent years and large pharmaceutical companies such as Novartis have stated their desire to move towards targeting smaller markets/populations instead. This is illustrated in fig. 4 and as the annual weight of drugs produced increases, there is a decrease in variety of drugs produced, a natural result of the intended consumer and the condition/disease treated. Targeting smaller markets can include reformulations or combinations of existing drugs that can be customised or adjusted to fill the needs of sub-groups or niches. On an even smaller scale, medication for rare diseases and personalised medication can be developed. A move away from the so-called "blockbuster" model of creating revenue and embracing a model of a broad range of more specialised treatments produced at a lower scale allows for the possibility of inkjet printing to be used at every point from drug discovery to packaging and distribution (1, 2).
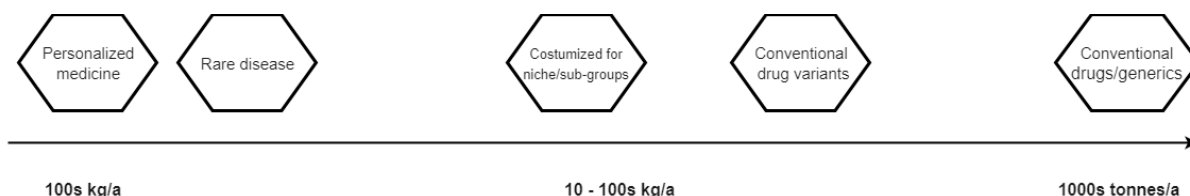
Figure 4. Overview of industrial production of medicine by volume related to the markets ranging from mass production to individualised doses. Adapted from (2)

High throughput screening (HTS) is an invaluable process for the drug discovery process where it can be used to test and analyse hundreds to thousands of samples. The large degree of automatisation, small scale of sample and operation of HTS combined with characteristics of IJP such as accuracy of drop volume and deposition make the application of IJP for HTS suitable. IJP also a suitable technology for use in genomics and combinatorial chemistry and this suitability has been shown by previous studies (16, 17, 18)

5.5 Personalisation of Medicine

The personalisation of medicine is an area in which IJP has major implications; certain populations and disorders require dosages that are not mass produced and cannot be created via splitting a commercially available tablet. Current means of manufacture do not make small scale production feasible economically or practically and current delivery methods are sub-optimal in regards to the specific needs of the target population. The latter point's relevance is made especially clear by the example drug Prozac, where 54% of the trial population showed an adequate response at a dose of 5 mg with fewer side effects and a lower dropout rate than the chosen dose of 25 mg - which 64% of the trial population responded to. Atenolol was initially released in a dose of 100 mg. This was reduced in 1980 and subsequently again in 1989 (19). Current means of personalising dosage include splitting tablets and oral formulations but these are not without issue. IJP can not only allow for the fabrication of a personalised dose but also for a more suitable delivery method.

Guidelines from the European Medical Agency can be an important framework for selecting suitable administration forms, especially for paediatric populations (19, 20). Paediatric and geriatric populations present unique challenges to formulation and

administration of medicine. These issues include: issues with swallowing due to age or due to factors associated with ageing such as loss of muscle tone and control, poor salivary production or quality, Parkinson's disease, strokes, dementia, cancer; physiological differences in organ function or in quality. It is not infrequent that the required dose can change within a relatively short time frame due to decreasing organ function or metabolic and physiological factors. The operation of delivery systems such as inhalers or sprays is dependent upon the skills of the user and may pose issues in the elderly. Excipients must be chosen carefully for paediatric populations due to potential differences in metabolism. Certain excipients commonly used in mass produced medications pose an increased risk of adverse effect in paediatric and geriatric populations. Adverse reactions can also have a greater effect on the general health of these vulnerable groups (22). Polypharmacy can complicate this and issues related to this frequently result in adverse drug related events and hospital admissions (23). These issues can be minimised with personalised doses that allow for closer adherence to the therapeutic index of a drug for an individual. This has been shown in Studies (6) and (13) where manipulation of ink formulation or deposition geometry altered release rates. Further control can be achieved by the use of enteric coated capsules in which a printed substrate can be placed.

Stability of printed preparations is an important consideration; studies reviewed that tested physical and chemical stability did so for a period of circa 30 days (19, 24). This makes the stability suitable for administration at point of production and shortly after but makes storage of large supplies or longer deliveries unsuitable. Thus it is currently a limiting factor for mid-scale production. On-site production at pharmacies or hospitals is not affected by a short shelf-life and as noted in Study 1, the ink may prove to be stable chemically and physically for years allowing for remote production, delivery and storage of inks (7, 24).

5.6 Microneedles

Microneedles have been heavily explored as a means to overcome several issues that accompany traditional vaccines such as thermostability, transport issues, hazardous waste, and fear/pain. Traditional microneedle technology involves a solid base of microneedles on which an API solution is coated. Coating of such microneedles allows for easier drug delivery and further developments have led to the design of hollow microneedles, which allow for an infusion, and dissolvable polymer microneedles encapsulating APIs. Controlled release rate can be achieved by altering ink formulation (15). Piezoelectric inkjet printing has shown to be a reliable technique resulting in an even deposition of polymer and/or API into a mould. Piezoelectric IJP allows for a further minimisation of waste - from utilising a higher drug load to production waste and finally sharps waste due to the dissolvable nature of moulded microneedles. The economic benefits that are possible through the use of IJP include reflect advantages for all stages of production and distribution.

Insulin is traditionally administered by needle by the patient but has a high degree of compliance issues as a result of this. Patients showing compliance often show other issues such as anxiety surrounding injection or management of the condition, or overuse of injection sites. Microneedles have been explored as an option for insulin delivery and by the use of differing polymers an acceptable release rate of insulin was achieved in Study 9, where a suitable insulin dose was released within 30 minutes, comparable to conventionally injected insulin. Optimised ink formulation resulted in complete, timely drug load release - an important aspect for hormones such as insulin and it was therefore considered suitable (14). The viability of dissolvable microneedles as a vaccination method was explored using a seasonal influenza vaccine (26). Inkjet printing has also shown suitability in the coating of moulded microneedles with the poorly soluble and antifungal agent voriconazole and a similar study showed similar success with itraconazole (27, 28).

Study (15) demonstrated the possibility of a multi-drug regime for the treatment of cancer being administered via microneedle technology. 5-fluororacil, curcumin and cisplatin were used with a SOL polymer. SOL appears very suitable in this capacity, possibly due to it being amphiphilic and acting as a solubiliser to increase bioavailability (15). This illustrates a further advantage of microneedle technology: the APIs used are traditionally administered by infusion, a lengthy process which can negatively affect the quality of life of patients - the use of microneedles could therefore improve that.

5.7 Poorly Soluble Drugs

Molecules with poor aqueous solubility represent a large proportion of abandoned drugs due to issues in production and slower *in vivo* dissolution rates. Study (8) showed the possibility of using IJP technology as a means of overcoming solubility challenges presented by the poorly soluble drug piroxicam. The use of IJP technology was shown to be preferable to an alternative production method. It was noted that the possibility of a suspension was considered although it was noted that agglomeration and precipitation were risks, as was nozzle clogging. It has been noted in study (7) that particles in a suspension that measure under 5 µm are more suited to printing and that nanosuspensions have considerably higher stability. The crystalline structure of folic acid was accounted for as it affects the stability of the ink, dissolution, bioavailability, and solubility. Solid amorphous APIs are preferable given their increased rate of dissolution but are less thermodynamically stable than crystal polymorphs. The carrier fluid and excipients can influence the crystalline structure of APIs and are an important aspect in ink formulation. This has been noted experimentally where thermally inkjetted prednisolone was detected in two polymorphic forms in the final product as opposed to a single polymorphic form in the starting material (29, 30). Study (11) used the model drugs paracetamol, caffeine, and theophylline to demonstrate how substrate penetration affected crystallisation of the API and how this can be taken advantage of when selecting a suitable substrate for printing poorly soluble APIs to increase their bioavailability and rate of dissolution.

5.8 Drawbacks of Inkjet Printing

The formulation of an ink that is suitable both for printing and as an effective medicine is something that requires careful consideration as to the chemistry and interactions of the API in relation to excipients, ancillary equipment, and regulatory guidelines. Practical issues regarding nozzle blockage and viscosity are paramount if the ink is to be suitable (1, 2, 3, 19). The concentration of API has been required to fall within therapeutic indexes and release profiles whilst concurrently maintaining optimal viscosity and printability. This has led to challenges in maintaining printability at the high concentrations required for therapeutic doses. However, this limitation may be less of an issue considering the generally lower doses needed for tailored doses in paediatric and geriatric populations. A focus on potent low dose APIs is also present. Concerns regarding the ethics of personalised medicine in practice relate to potential issues with equal access to medication that is optimised to the individual. Unless personalised medication was heavily subsidised or reserved for severe cases there is a risk that it benefits those who have the economic means to pay for a better standard of care - an especially valid concern outside of European countries where medication may already come at a steep cost (31, 32).

5.8.1 Carrier Fluid

Water, ethanol, DMSO and acetone are at present the most popular choices for carrier fluid during ink formulation. The carrier fluid may have several roles in the ink, such as a solvent for the API, an immiscible carrier phase should the ink be a colloidal dispersion, or as an element for controlling evaporation of the ink so that the API takes the preferred

morphology. Previously abandoned or difficult drug candidates may have the potential to be revisited due to the flexibility of IJP and ink formulation and the underlying technique. Good solubility is necessary as it allows for higher uptake and bioavailability (1, 2, 3, 8). However, the potential usage of inkjet technology as a means to personalise medicine and tailor it to an individual or group is not without issue - ranging from adequate dosage to storage.

Regulatory factors may determine printing substrate, choice of excipients, and administration method (19). The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) sets guidelines concerning the amount of ethanol that is allowed in a certain formulations for specific populations, which could limit the usage of ethanol as a solvent and in turn limiting potential APIs. The ICH is an international non-profit organisation and committee featuring the European Commission, the American Food and Drugs Agency, Japan's Ministry of Health as the founding regulatory body members. The ICH was formed in 1990 as a response to the increasingly international nature of the pharmaceutical market and industry and the lack of trans-national regulations. Safety, quality and efficacy are the focal points for the ICH's regulatory framework and guidelines. Several countries have translated the ICH's regulatory framework and guidelines into a legal capacity. The goal of the ICH's guidelines is to harmonise national technical regulations and requirements on the development, production, and research of pharmaceutical products (33).

5.8.2 Ancillary Equipment

Research on the interaction of the API, carrier fluid with ancillary equipment falls short. These issues are raised in detail in the literature where each aspect is considered and technical difficulties and issues that can arise in bulk handling of pharmaceutical liquids are noted (19). Adsorption of API or non-selective binding of proteins/enzymes to the ink reservoir is an issue for consideration when using a large reservoir of slow-moving fluid - such as would be necessary on an industrial scale. Despite each nozzle only ejecting a minute volume in the region of 10 - 500 pL, the ancillary equipment used would be required to be on a much larger scale. Leaching of elements from ancillary equipment into the ink may cause issues related to formulation as viscosity may be affected. Bioactive elements from ancillary equipment leaching into the ink is a very real concern, as noted in the literature where an immune response was observed after leaching from ancillary equipment into erythropoietin occurred (34). Every new piece of equipment added requires careful examination and consideration as to how it may interact with the pharmaceutical ink and vice versa. Besides issues with bioactivity, leaching of elements into the ink may result in formulation issues aside from viscosity as the contaminated ink may exhibit different behaviours in regards to swelling and thus degrading the fluid path. This is already a concern in the choice of carrier fluid as water, DMSO, dichloromethane, and ethanol each have particular challenges in this regard. Should nanosuspensions or nanoemulsions be used, flocculation, settling, creaming, aggregation, and Ostwald ripening are feasible and could account for issues of homogeneity/concentration and nozzle blockage (1, 2, 3). Little mention of Good Manufacturing Practice (GMP) occurs within the literature which may lead to concerns regarding the application of GMP outside of experimental research. Concerns regarding sterility of equipment is raised within the literature (19).

5.8.3 Ink Formulation

Formulation of the ink is of importance as interaction with ancillary equipment and sub-par stability can lead to issues and must occur with foresight to the intended equipment. A high concentration of low potency API may result in lower printability due to the increased risk of nozzle blockage resulting from flocculation, settling, creaming, aggregation, or Ostwald ripening. The current research is largely focused on small molecules and biopharma, resulting in a wide variety of target APIs for IJP being

explored, allowing for future investigation as to the "families" of API that may be suitable and base ink formulation for them. As shown by Studies 7 and 9, printing proteins can overcome many of the traditional issues surrounding administration; the usage of IJP may be a viable solution to that, given that the selected enzymes and peptides were shown to retain activity after processing (12, 14).

As a result of their origin and function, proteins and peptides are often highly specific and well-tolerated. A high degree of specificity involves a higher degree of precision in dosing. The economic benefits have a huge potential due to shorter approval times and patent protection with a larger scope. Proteins and peptides have long posed a challenge pharmaceutically as whilst they may possess desirable pharmacological properties they are unable to be processed and administered via oral delivery routes. This is largely due to the acidic environment of the stomach and the presence of enzymes leading to cleaving and denaturing, rendering them unable to pass into the bloodstream in an active form. Protein or peptide activity is often dependent upon the tertiary structure and should deformation or denaturation occur the function is lost. The large molecular size of many proteins is another factor in their poor permeability and bioavailability; molecules over 500 - 700 Da show a decrease in both factors. Insulin has a molecular mass of 5808 Daltons and etanercept (Enbrel®) has a molecular mass of 51235 Daltons. Many proteins and peptides undergo extensive first-pass metabolism, rendering them unavailable for their intended purpose. Conventional methods of delivery are therefore largely limited to infusion or injection as this bypasses first-pass metabolism and stomach and intestinal environments (12, 35, 36). Study 9 showed the feasibility of not only printing insulin by piezoelectric inkjet technology but also that insulin can be administered by microneedles with good *in vitro* diffusion rates (14).

There are certain additional issues presented during the printing of bioinks including frothing of the ink and unwanted enzymatic activity (27, 34). Formulation and rheology of the ink is of vital importance but the protein component and necessary stabilisers may result in an ink with sub-optimal Z-numbers (1). One solution to the sub-optimal viscosity of the ink that was explored was the usage of a higher voltage and lower back pressure. This resulted in fewer satellite drops and a monodisperse drop formation but lower API integrity was observed, possibly due to higher shear forces. Issues with bovine insulin deformation as a result of shear forces has been noted. This appears linked to ink formulation as no shear force-linked issues were noted in study 9 (14, 37).

5.9 Quality Control
The use of IJP technology on any scale poses issues with quality assurance and control. Randomised testing of batches is unsuitable for smaller scale production - especially for the production of personalised medication. The addition of fluorescent parties or dyes to the ink may be a viable solution for some types of API. Certain dyes may prove unsuitable of specific individuals. An *in situ* spectrometer could be an alternative for control of correct dose during production. Several experimental methods have been used to characterise concentration and presence of APIs using various parameters and may be applicable in mid-scale production but may not to small scale production in hospitals or pharmacies. Some of these methods, such as ultraviolet-visible spectroscopy, may prove to be suitable for use in hospital settings. Limitations of viable characterisation methods in hospitals and pharmacies are tied to economic, time and storage constraints. The EMA encourages drug manufacture to include quality-by-design, bypassing the need of heavy characterisation post-production. Commercial printers may be fitted with optical and/or electrostatic sensors which detect failure of a nozzle to fire, with backup systems in case of failure. This is a more suitable method for small-scale production in combination with an ink containing a dye for control of dosage or gravimetric control of weight, assuming this conforms to local guidelines (38).

6. Conclusion

This study has shown through a review of experimental studies that IJP can successfully be used on a small scale to produce tailored doses of proteins and poorly soluble drugs for oral or transdermal administration. As both proteins and poorly soluble drugs can be printed with success, IJP may offer an attractive new research and development opportunity to revisit previously abandoned APIs or allow for improved formulation and administration of existing APIs. Controlled release can be tailored to individuals or treatment regimes by manipulating the deposition and ink formulation - allowing for a high degree of precision in choosing and maintaining a dose. IJP is not an established technology and it is therefore difficult to foresee how the technology may develop but it has shown great advantages experimentally in the production of oral doses and coating medical devices. Microneedles are a more established technology, featuring in clinical trials and it is therefore considered likely that IJP will be utilised more in the production of microneedles once progress has been made in ink formulation. Ink formulation is perceived as one major area that needs further investigation before IJP can be utilised outside of experimental research. Current exploration into IJP has been on an experimental level and focused on future small to mid scale manufacture of medicine; it is therefore considered likely that the future of IJP will be focused on production in hospitals and pharmacies for individuals or niche groups with specific needs. The cost of the production and determining personalised aspects would likely lead to the technology being reserved for a small amount of individuals rather than it becoming a mainstream technology available to the masses. Dose quantification and characterisation are also considered prohibitory factors to the technology being used outside of research until more accessible and effective methods are developed.

7. Acknowlegements

8. References

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