3D Printing of five-in-one dose combination polypill with defined immediate and sustained release profiles

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Abstract

We have used three dimensional (3D) extrusion printing to manufacture a multi-active solid dosage form or so called polypill. This contains five compartmentalised drugs with two independently controlled and well-defined release profiles. This polypill demonstrates that complex medication regimes can be combined in a single personalised tablet. This could potentially improve adherence for those patients currently taking many separate tablets and also allow ready tailoring of a particular drug combination/drug release for the needs of an individual. The polypill here represents a cardiovascular treatment regime with the incorporation of an immediate release compartment with aspirin and hydrochlorothiazide and three sustained release compartments containing pravastatin, atenolol, and ramipril. X-ray powder diffraction (XRPD) and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) were used to assess drug-excipient interaction. The printed polypills were evaluated for drug release using USP dissolution testing. We found that the polypill showed the intended immediate and sustained release profiles based upon the active/excipient ratio used.

Keywords

3D printing; Polypill; Sustained release; Immediate release; Personalised medicine

Chemical compounds studied in this article

Aspirin (PubChem CID: 2244); Hydrochlorothiazide (PubChem CID: 3639); Atenolol (PubChem CID: 2249); Pravastatin sodium (PubChem CID: 16759173); Ramipril (PubChem CID: 5362129)

1. Introduction

The use of multiple medications to control complex diseases such as cancer and heart diseases is an increasingly used therapeutic strategy [1, 2]. Each active pharmaceutical ingredient is traditionally administered via a separate dosage form [2]. This is inconvenient, can lead to errors in medication and presents significant patient compliance issues [2, 3]. Combining multiple actives into a single tablet with appropriate release profiles and doses (potentially optimised for individuals) is an attractive alternative [3-5].

The term "polypill" refers to a tablet that is composed of a combination of several medicines [5], and has been previously studied as a concept to treat and prevent cardiovascular disease and high blood pressure [6-9]. This polypill (in fact a capsule) manufactured by Cadila Pharmaceuticals Limited under trade name of Polycap^{TM} is the only polypill formulation commercially available [7, 8, 10]. Cardiovascular disease is the most common cause of death globally and requires managing as a chronic condition in many people during large portions of their lifetime [11]. Based on previous work, we suggest that additive manufacturing or 3D printing is potentially well suited to producing a multicomponent polypill formulation [4, 8, 9]. As an approach 3D printing also offers the opportunity to produce personalised medicines and is adaptable to a distributed manufacturing model [4]. The freedom to form specific geometries in comparison to the restrictions of traditional tableting via powder compression can be used to separate incompatible substances and to enable different release rates using shape and size as well as excipient manipulation [4, 12]. Here we have designed a 5-component polypill based upon the currently available “polycap” commercial formulation with three sustained release compartments containing pravastatin, atenolol, and ramipril, which were physically separated by a hydrophobic cellulose acetate shell designed to act as a permeable carrier, and covered with an immediate release aspirin and hydrochlorothiazide compartment (Fig. 1.). Atenolol is a beta-blocker agent which is used to treat hypertension and also prevent and/or treat heart attack [13]. Hydrochlorothiazide is a thiazide diuretic used to prevent absorption of too much salt and to treat oedema or fluid retention in individuals with congestive heart failure, kidney disorder, and liver

cirrhosis [14]. Ramipril is an angiotensin converting enzyme (ACE) used for treatment of hypertension and congestive heart failure which improves heart function after a heart attack [15]. Aspirin is an antiplatelet used to reduce the risk of blood clotting and reduce heart attacks or strokes [16]. Pravastatin is a 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors used to reduce blood cholesterol and triglycerides in hyperlipidaemic patients and lower rates of strokes and heart attacks [17].

3D printing is a process used to fabricate 3D objects by laying down successive material layers in different shapes taken directly from a digital file [18]. There has been a significant recent growth in interest of 3D printing as a tool in pharmaceutics and personalised medicine [19-23]. For example, a heat based fused deposition modelling 3D printer (> 200 °C) has been used to extrude 5-aminosalicylic acid (5-ASA, mesalazine), and 4-aminosalicylic acid (4-ASA) and prednisolone loaded poly (vinyl alcohol) (PVA) filaments and produce simple solid tablets [19, 24]. However, this approach would not be suitable generally due to the possibility of heat induced degradation of thermally sensitive drugs. Also, there are not many reports of printing a single drug formulation with multiple release mechanisms [4, 25]. Katstra et al. employed multi-steps 3D printing to deposit chlorphenaramin maleate (antihistamine used in the prevention of symptoms of allergic conditions such as rhinitis and urticaria) as a binder onto powdered excipients (the amount of drug deposited was 5.45 mg) [25, 26]. However, issues such as ink bleeding, migration, and capillary effect due to drug/binder oversaturation are difficult to avoid for printing of larger drugs doses such as 500 mg of paracetamol or ibuprofen [25, 26]. Problems with this approach include long drying times (in excess of 50 hours) and high friability (> 1 %) of the resultant tablet [25, 26].

To address the above mentioned issues of drug degradation and the complexity in published 3D printing processes we have employed a 3D extrusion system operated at room-temperature to manufacture a polypill capable of delivering the five drugs via two predictable release mechanisms. A hydrophobic cellulose acetate shell was first extruded then the active drugs atenolol, pravastatin, and ramipril were mixed with a hydrophilic matrix (HPMC) and extruded in to the segmented

compartments of cellulose acetate to form sustained release compartments. Aspirin and hydrochlorothiazide were mixed with a disintegrant; sodium starch glycolate and other excipients and extruded directly on the top of the sustained release compartments, to give an immediate release compartment. A series of raised dots were also printed onto the top of the tablet to facilitate identification of the formulation both visually and by touch, the composition of these was the same as the upper "immediate release" layer. The printed tablets were tested for drug release and drug-excipients interaction using United States Pharmacopeia Convention (USP) Type I apparatus dissolution tester, X-Ray Powder Diffraction (XRPD), and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR).

2. Materials and methods

2.1. Materials

Ramipril and pravastatin sodium were supplied by Kemprotec Limited (Cumbria, UK). Atenolol, aspirin, and hydrochlorothiazide, polyvinylpyrrolidine (PVP) and lactose were supplied by Sigma–Aldrich (Gillingham, UK). D-mannitol 99 % was purchased from VWR International Ltd. (Leicestershire, UK). Sodium starch glycolate (Primojel®) was kindly supplied as a gift from DFE Pharma. Hydroxypropyl methylcelloluse (HPMC K100M CR) (Methocel TM) was a gift from Colorcon®. Milli-Q water (resistivity 18.2 MΩ cm) was used for all formulations and solutions. All other reagents were of either HPLC or analytical grade.

2.2. Methods

2.2.1. Design of polypill

A segmented tablet strategy was chosen to ensure that the actives were separated and could achieve the desired independent control of their release (Fig. 1). This concept provides flexibility in production of a 3D printed polypill with tunable drug release based on modifying the drug loading and excipient composition in the separate parts of the formulation. The dimensions of the polypill were selected according to the drug loading in respect of selected excipients (5.85 mm (height) × 6 mm (radius)). The geometry of the polypill was designed using a 3D drawing package (BioCAD,

regenHU Villaz-St-Pierre, Switzerland). The combined drugs and their loadings in the polypill described in the experimental set up were adapted from clinical studies based on assessment of effect combination therapy on healthy middle aged individuals with one or more risk factors [8].

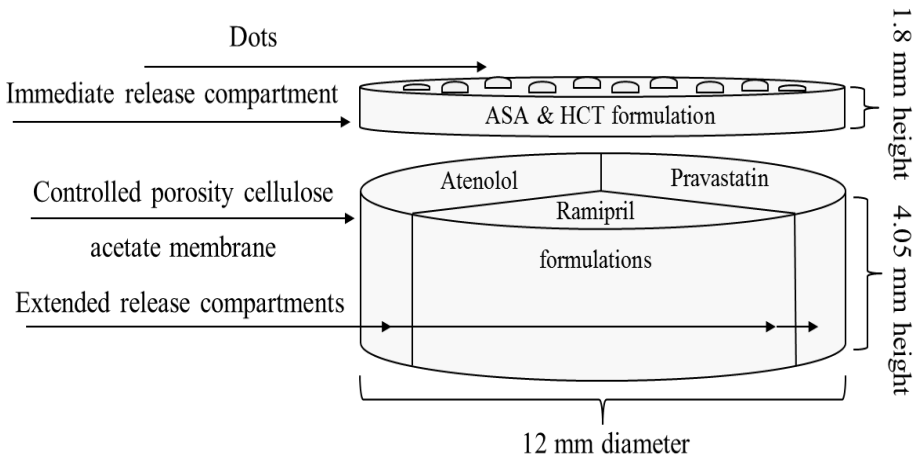

Fig. 1. Schematic structural diagram of the polypill design, showing the aspirin and hydrochlorothiazide immediate release compartment and atenolol, pravastatin, and ramipril sustained release compartments.

2.2.2. Materials for printing of polypill

All powders were mixed using a mortar and pestle for 15 minutes. The printable paste used to form the barrier for the sustained release actives was prepared by mixing 3.15 g from the blended powder mixture; cellulose acetate (hydrophobic membrane/shell), D-mannitol (a filler), and polyethylene glycol (PEG 6000) (plasticizer) with 1.7 ml of the binder (acetone and dimethyl sulfoxide (DMSO)) until a smooth homogenous paste was achieved according to the formulae in Table 1. DMSO was used to increase the boiling point of the binder system and avoid nozzles blockage due to acetone evaporation (low boiling point) during the extrusion process. We used acetone/DMSO at a ratio of 3:1 v/v. The volume of DMSO per tablet was approximately 28 µl, equivalent to 30.8 mg (3080 ppm) which is considered a very small volume/quantity in respect to DMSO human toxicity and was within the limit of the class 3 solvents (5000 ppm/day) which stated in ICH Harmonised Tripartite Guidance [27-31].

Table 1. The weight percentage composition of various ingredients in cellulose acetate shell for sustained release formulation in feed stock.

Ingredients	Function	Coating (% w/w)
Cellulose acetate	Hydrophobic shell	22.64
D-mannitol	Filler	62.26
PEG (6000)	Plasticizer	15.10

Powders of atenolol, pravastatin, and ramipril were separately blended using a mortar and pestle for 15 min with the required excipients, to ensure homogeneous powder blend. Ultra-pure water was added to the powder and mixed as above method according to the formulae shown in Table 2. The immediate release layer was composed of aspirin and hydrochlorothiazide (active ingredients), sodium starch glycolate (disintegrant), and polyvinylpyrrolidone (PVP K30) (binder). The powder was blended and mixed with ultra-pure water to form a smooth and soft paste according to the formulae shown in Table 3.

Table 2. The weight percentage composition of various ingredients in atenolol, pravastatin, and ramipril formulation feed stock for the sustained release compartments of the polypill.

Ingredients	Function	ATEN-HPMC* (15 % w/w)	PRA-HPMC** (15 % w/w)	RAM-HPMC*** (15 % w/w)
Atenolol	Active ingredient I	30.00	----	----
Pravastatin	Active ingredient II	----	20.00	----
Ramipril	Active ingredient III	----	----	15.00
HPMC 2208	Hydrophilic matrix	15.00	15.00	15.00
Lactose	Filler	55.00	65.00	70.00

*ATEN = atenolol, **PRA = pravastatin, and ***RAM = ramipril

Table 3. The weight percentage composition of various ingredients in aspirin and hydrochlorothiazide immediate release formulation feed stock for the immediate-release compartment of the polypill.

Ingredients	Function	ASA_HCT-IR* compartment
Aspirin	Active ingredient	28.62
Hydrochlorothiazide	Active ingredient	5.86
Sodium starch glycolate	Disintegrant	55.18
Polyvinylpyrollidine K30	Binder	10.34

*ASA = acetylsalicylic acid (aspirin), HCT = hydrochlorothiazide, and IR = immediate release

2.2.3. 3D based extrusion printing process

All the pastes were loaded into separate ink cartridges for extrusion through a 500 µm print tip. The cellulose acetate shell was first extruded (no drying step needed), followed by extrusion of sustained release pastes inside the segmented cellulose acetate shell. The immediate release layer was then extruded on the top to cover the sustained release compartments, with the addition of identifying raised dots to illustrate the ability to provide integrated identification (visual and feel based) of the polypill (Fig. 2). The total printing time was 25 min followed by being placed in a vacuum dryer at 40 °C for 24 hours for complete drying.

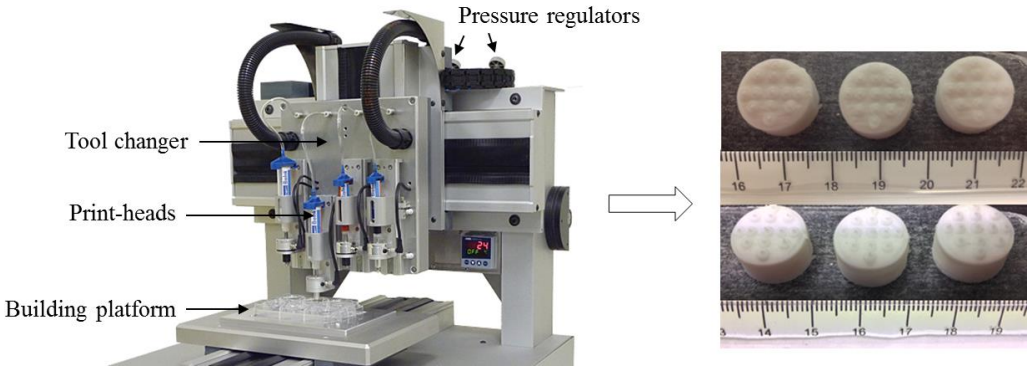

Fig. 2. Photograph of regenHU 3D printer (left) [32], and image of multi-active tablet (right) (5.85 mm (height) × 6 mm (radius) composed of sustained release compartments (bottom), and immediate release dotted compartment (top).

183 *2.2.4. Dissolution studies*

184 A HP Agilent 1050 HPLC equipped with an ACE C18-AR analytical column (100 mm x 4.6 mm)
185 with 5 µm particle size was used to analyse dissolution release media for drug content. The auto-
186 sampler was set up to make 40 µl injections, every 25 minutes. The flow rate of the mobile phase was
187 1 ml / min, the column temperature was 40 °C and the UV detection wavelength was 215 nm. The
188 mobile phase (acetonitrile and water containing 0.1 % v/v of trifluoroacetic acid) was degassed and
189 filtered through a 0.45 µm membrane filter. A mixture of actives (75 mg of aspirin, 12.5 mg of
190 hydrochlorothiazide, 25 mg of atenolol, 20 mg of pravastatin, and 5 mg of ramipril) was dissolved in
191 the dissolution medium and separated using the above HPLC method (Supplementary data, Appendix
192 A, Fig. S.I. 1).

193 *In vitro* drug release studies of the 3D printed polypill were performed using USP Type I apparatus
194 (rotation speed at 50 rpm, 900 ml phosphate buffer, pH 6.8 containing 0.5 % of tween 80 (v/v) as the
195 dissolution media at 37˚C ± 0.5˚C). 5.0 ml samples were withdrawn at 5, 15, 30, 60, 120, 240, 360,
196 480, 600, 720 min. The samples were centrifuged and a small volume from the supernatant was drawn
197 and filled into HPLC amber glass vials. The samples were kept at 4 °C (to decrease drug degradation)
198 until tested. Drug dissolution studies were conducted in triplicate and the average of percentage of
199 cumulative drug release as a function of time was determined.

200 *2.2.5. X-Ray Powder Diffraction (XRPD)*

201 The XRPD patterns of pure atenolol, pravastatin, ramipril, hydrochlorothiazide, and aspirin and their
202 formulations (immediate and sustained release formulations) were obtained at room temperature using
203 an X'Pert PRO (PANalytical, Almelo, Netherlands) setup in reflection mode using Cu $K\alpha_1$ (lambda =
204 1.54 Å) operating in Bragg–Brentano geometry. The generator voltage was set to 40 kV and the
205 current to 40 mA and the samples were scanned over 2θ range of 5˚ until 30˚ in a step size of 0.026˚.

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207

208 *2.2.6. ATR-FTIR*

209 In order to investigate possible interactions between the actives and the selected excipients in their
210 formulations, infrared spectra of pure active ingredients and their formulations were obtained using an
211 ATR-FTIR (Agilent Cary 630 FTIR) spectrometer.

212 **3. Results and discussion**

213 *3.1. In vitro drug dissolution*

214 Dissolution data from the polypill (Fig. 3) show that more than 75 % of the aspirin and
215 hydrochlorothiazide were released within the first 30 minutes. This drug release is attributed to the
216 inclusion of the disintegrant, sodium starch glycolate, which rapidly absorbs water and swells leading
217 to rapid disintegration of this portion of the polypill and fast drug release. The same figure also shows
218 that atenolol, pravastatin, and ramipril displayed sustained release over a period of 720 min as
219 required; with 69 %, 81 %, and 66 % released respectively. This release is consistent with the effects
220 of rapid hydration of the HPMC leading to a gel like formation and swelling to form a hydrophilic
221 matrix that slows drug release and also to the presence of the permeable cellulose acetate shell which
222 is expected to retard drug release [33-35].

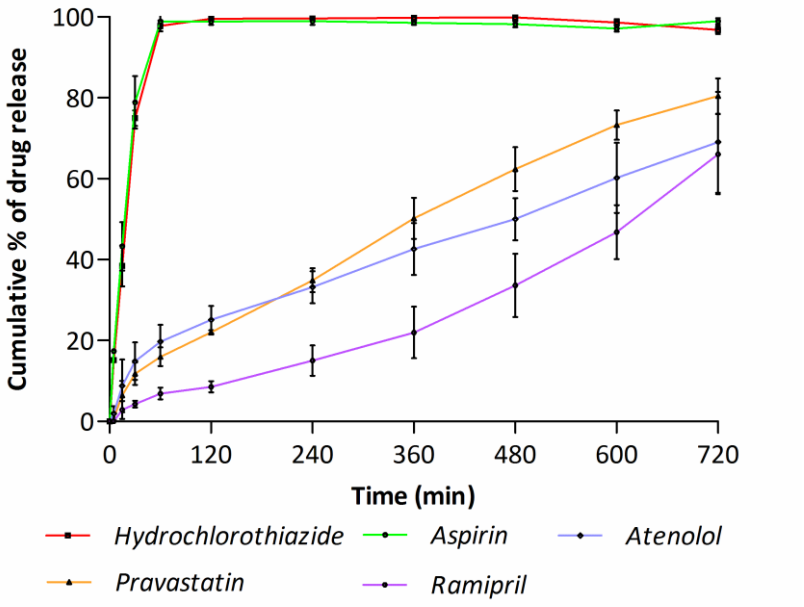

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Fig. 3. *In vitro* cumulative drug release profile of each drug from the five drug-loaded compartments of the polypill.

These data clearly illustrate the potential to achieve different drug release profiles from the same tablet for different drugs. As the three drugs that require sustained release are in separate compartments this also clearly shows the opportunity that 3D printing provides to vary loading and the fine detail of each drug release.

3.2. Physical characterisation of immediate and sustained release formulations

3.2.1. XRPD

XRPD data were collected on the pure as-received drugs before printing, and on the mixed formulations (immediate and sustained release formulations) containing the drugs after printing, in order to investigate any changes in physical form on printing (Figs. 4 and 5). All as-received materials exhibit multiple sharp Bragg peaks in their XRPD patterns related to their crystalline nature, except pravastatin which exhibits no sharp Bragg peaks indicating that it is exist in an amorphous state. The patterns for the crystalline materials match those reported in the Cambridge Structural Database (Fig. 4) [36-39]. After formulation and printing, the Bragg peaks for ramipril, aspirin, hydrochlorothiazide, and atenolol are still present, with peaks also observed as expected from the excipients. For example, the appearance of a sharp peaks due to lactose are clearly visible at 20, 19, and 18 degrees 2-theta for the pravastatin, ramipril, and atenolol formulations, and the broad feature due to HPMC is visible at around 20 degrees 2-theta for the atenolol formulation. There is therefore no evidence of a change in physical form for the drugs in these three formulations. The situation is comparable for pravastatin, for which no obvious Bragg peaks from pravastatin are visible in both as-received and in the final formulation.

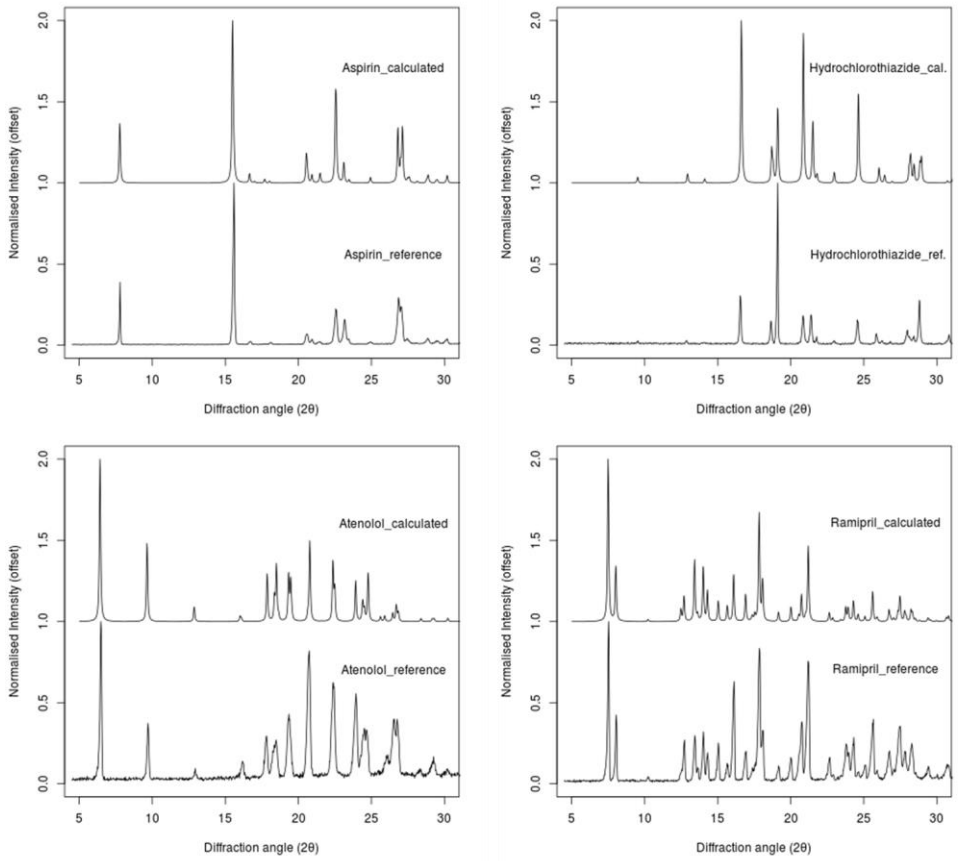

Fig. 4. XRPD patterns of the calculated and reference (measured) aspirin (top-left), hydrochlorothiazide (top-right), atenolol (bottom-left), and ramipril (bottom-right).

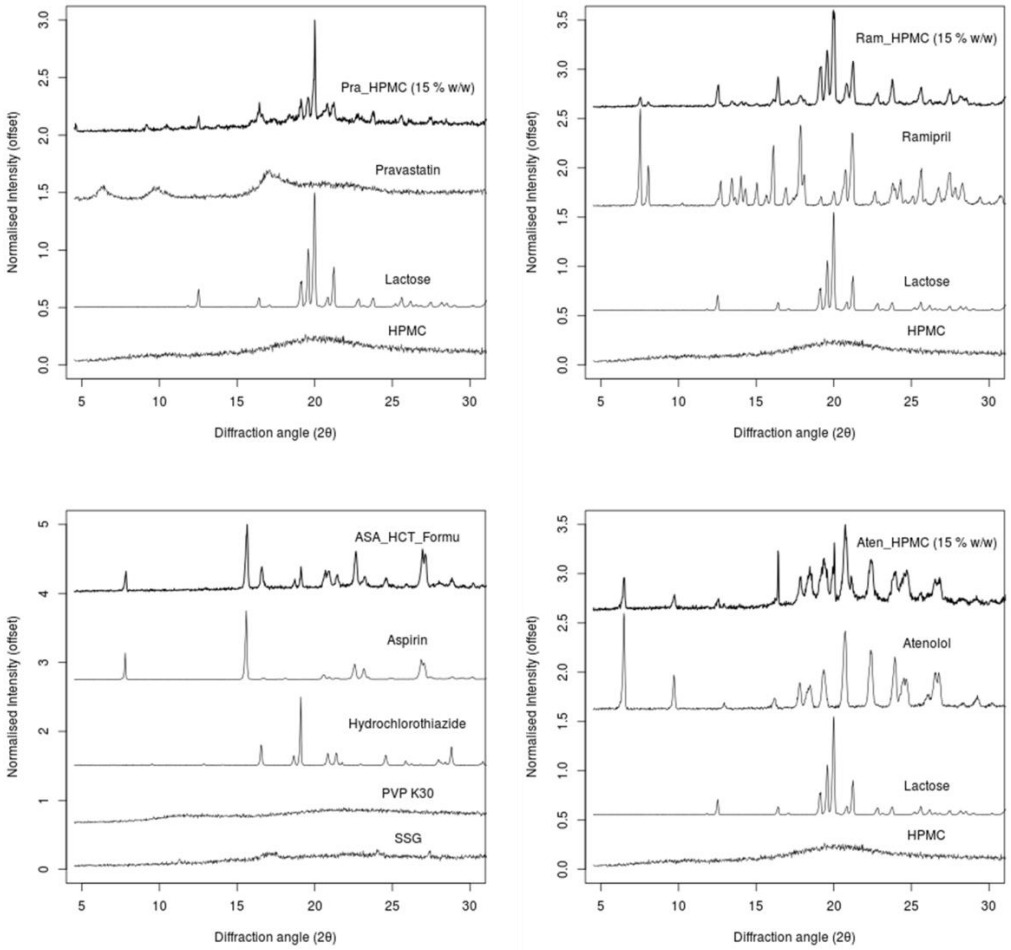

Fig. 5. XRPD patterns of *Pra-HPMC (15 % w/w)*, pure pravastatin, lactose, and HPMC (top-left), *Ram-HPMC (15 % w/w)*, pure ramipril, lactose, and HPMC (top-right), *ASA-HCT-Formu*, pure aspirin, pure hydrochlorothiazide, (polyvinylpyrrolidine) PVP k30, and sodium starch glycolate (bottom-left), and *Aten-HPMC (15 % w/w)*, pure atenolol, lactose, and HPMC (bottom-right).

3.2.2. ATR-FTIR

The major diagnostic infrared peaks of the actives did not change within the formulations as compared to control spectra of the drugs alone, indicating that there were no detectable interactions between the drugs and the selected excipients (Fig 6).

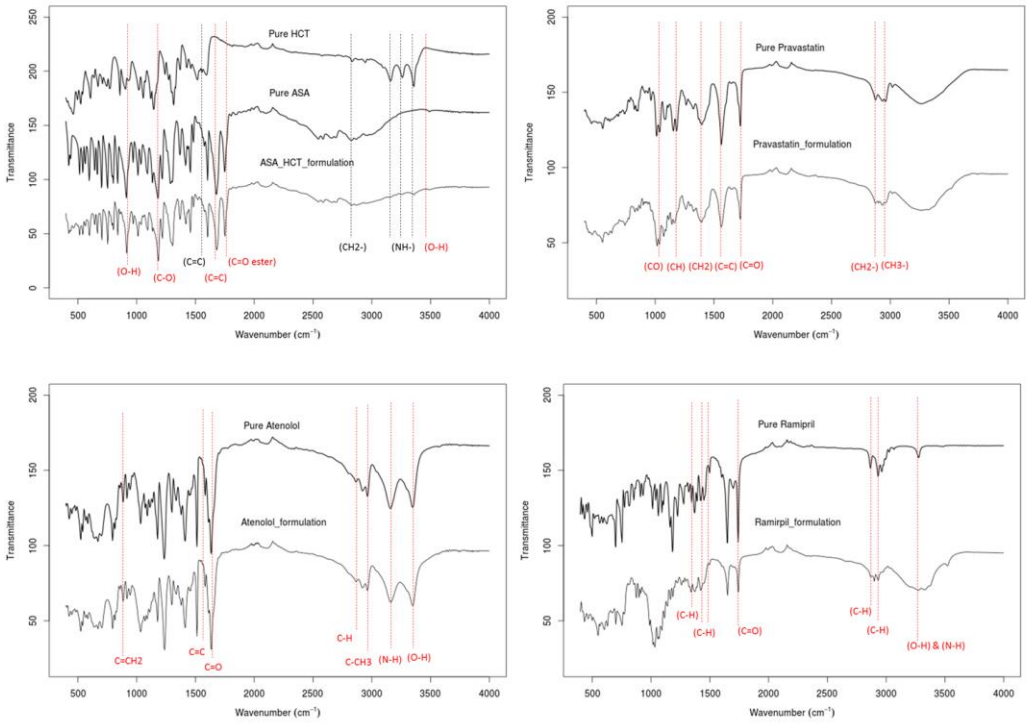

Fig. 6. FTIR spectra of pure actives; aspirin and hydrochlorothiazide (top left), pravastatin (top right), atenolol (bottom left), and ramirpil (bottom right) and their formulations.

It should be noted that all the polypills were printed to the expected size, mechanically stable, and can be handled readily without any loss of structural integrity.

A relationship has been shown between heart disease and renal failure and various modifiable risk factors, such as hypertension, dyslipidaemia, and platelet capacity [40-43]. Therefore, patients over 55 years old with one or more risk factors, including hypertension, obesity, and diabetes can benefit from a combined medications such as that demonstrated here that includes an antiplatelet, cholesterol and blood pressure lowering agents [44-46]. To use a combined medication approach successfully a number of challenges need to be considered, including; making the novel dosage form acceptable to health professionals and patients; formulation issues; additional cost; achieving regulatory approval; ensuring this becomes a first-line therapy (should be more effective and have no more side effects than drugs taken individually) [3]. The polypill demonstrated here addresses some of these issues, it is of an acceptable size and appearance for a patient, offers the prospect of increasing patient adherence

since only one tablet needs to be taken [4], and also should reduce risk of mistakes by patients who forget to take a certain medicine within a combination of different tablets [3]. Issues such as cost and regulatory approval are beyond the scope of the current work.

Furthermore, application of 3D printing in pharmaceutics could offer a flexible tool to tailor the combined drug doses according to the patient's needs. For example, control of tablet size and shape for children and elderly patient with difficulty handling tablets or swallowing, and printing 'specials' for tablets with allergies to certain excipients. We have demonstrated in this paper that a simple visual/tactile identifier can readily be added to 3D printed tablets to aid sight-compromised patient as well.

4. Conclusions

We have successfully demonstrated 3D extrusion printing of a novel complex geometry five-in-one polypill. We have also proved that the polypill is able to deliver five actives via two different and well defined release mechanisms: immediate and sustained release. The drugs were physically separated in the polypill to avoid incompatibility issues and allow maximum flexibility in manipulating the environment of each drug. XRPD and FTIR data were used to show that there was no detectable interaction between the drugs and the chosen excipients, and that our method of 3D printing did not lead to a detectable change in the physical form of the drugs (e.g. polymorphism, hydration etc). Such a combination of actives as used here has been shown to be important in prevention and treatment of cardiovascular diseases. The drugs combined as a polypill provide the prospect of improved adherence to such combination therapies due to the convenience of a single tablet and the potential to optimise and personalise dosages and release for each drug independently in such multi-drug dosage forms.

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References

[1] J. Gao, C. Chen, J.-X. Chen, L.-M. Wen, G.-L. Yang, F.-P. Duan, Z.-Y. Huang, D.-F. Li, D.-R. Yu, H.-J. Yang, S.-J. Li, Synergism and Rules of the new Combination drug Yiqijiedu Formulae (YQJD) on Ischemic Stroke based on amino acids (AAs) metabolism, Sci. Rep., 4 (2014) 1-11.

[2] S. Bangalore, A. Shahane, S. Parkar, F.H. Messerli, Compliance and fixed-dose combination therapy, *Curr. Hypertens. Rep.*, 9 (2007) 184-189.

[3] P. Sleight, H. Pouleur, F. Zannad, Benefits, challenges, and registerability of the polypill, *Eur. Heart J.*, 27 (2006) 1651-1656.

[4] S.A. Khaled, J.C. Burley, M.R. Alexander, C.J. Roberts, Desktop 3D printing of controlled release pharmaceutical bilayer tablets, *Int. J. Pharm.*, 461 (2014) 105-111.

[5] M. Lafeber, D.E. Grobbee, M.L. Bots, S. Thom, R. Webster, A. Rodgers, F.L. Visseren, W. Spiering, The Evening versus Morning Polypill Utilization Study: the TEMPUS rationale and design, *Eur. J. Prev. Cardiol.*, 4 (2013) 425-433.

[6] J.D. Spence, Polypill: for Pollyanna*, *Int. J. Stroke*, 3 (2008) 92-97.

[7] K.M. Carey, M.R. Comee, J.L. Donovan, A.O. Kanaan, A polypill for all? Critical review of the polypill literature for primary prevention of cardiovascular disease and stroke, *Ann. Pharmacother.*, 46 (2012) 688-695.

[8] S. Yusuf, P. Pais, R. Afzal, D. Xavier, K. Teo, J. Eikelboom, A. Sigamani, V. Mohan, R. Gupta, N. Thomas, Effects of a polypill (Polycap) on risk factors in middle-aged individuals without cardiovascular disease (TIPS): a phase II, double-blind, randomised trial, Lancet, 373 (2009) 1341-1351.

[9] J. Hippisley-Cox, C. Coupland, Effect of combinations of drugs on all cause mortality in patients with ischaemic heart disease: nested case-control analysis, *BMJ*, 330 (2005) 1059-1063.

[10] C.P. Limited. (2015). Polycap Retrieved 26/06/2015, from http://www.polycap.org/index.html

[11] D.M. Lloyd-Jones, E.P. Leip, M.G. Larson, R.B. d'Agostino, A. Beiser, P.W. Wilson, P.A. Wolf, D. Levy, Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age, Circulation, 113 (2006) 791-798.

[12] E. Lonn, J. Bosch, K. Teo, P. Pais, D. Xavier, S. Yusuf, The polypill in the prevention of cardiovascular diseases: key concepts, current status, challenges, and future directions, Circulation, 122 (2010) 2078-2088.

[13] J. Jia, C. Dong, W. Zhang, Y. Cui, J. Liu, Evaluation of pharmacokinetic and pharmacodynamic relationship for oral sustained-release atenolol pellets in rats, *J. Pharm. Biomed. Anal.*, 55 (2011) 342-348.

[14] N.A. VH, G. Niharika, P. Deepak, S. Nazan, S.A. Mohammed, Formulation design, characterisation and in vitro evaluation of bilayered tablets containing telmisartan and hydrochlorthizide, *Int. J. Bio.*, 1 (2013) 1-9.

[15] S. Shafiq, F. Shakeel, S. Talegaonkar, F.J. Ahmad, R.K. Khar, M. Ali, Development and bioavailability assessment of ramipril nanoemulsion formulation, *Eur. J. Pharm. Biopharm.*, 66 (2007) 227-243.

[16] A.T. Cohen, S. Imfeld, J. Markham, S. Granziera, The use of aspirin for primary and secondary prevention in venous thromboembolism and other cardiovascular disorders, *Thromb. Res.* , 135 (2015) 217-225.

[17] S.C. Halbert, B. French, R.Y. Gordon, J.T. Farrar, K. Schmitz, P.B. Morris, P.D. Thompson, D.J. Rader, D.J. Becker, Tolerability of Red Yeast Rice (2,400 mg Twice Daily) Versus Pravastatin (20 mg Twice Daily) in Patients With Previous Statin Intolerance, *Am. J. Cardiol.*, 105 (2010) 198-204.

[18] W. Liu, Y. Li, J. Liu, X. Niu, Y. Wang, D. Li, Application and Performance of 3D Printing in Nanobiomaterials, *J. Nanomater.*, 2013 (2013) 1-7.

[19] J. Skowyra, K. Pietrzak, M.A. Alhnan, Fabrication of extended-release patient-tailored prednisolone tablets via fused deposition modelling (FDM) 3D printing, *Eur. J. Pharm. Sci.* , 68 (2015) 11-17.

[20] C.L. Ventola, Medical Applications for 3D Printing: Current and Projected Uses, *Pharm. Ther.*, 39 (2014) 704-711.

[21] N. Scoutaris, M. Snowden, D. Douroumis, Taste masked thin films printed by jet dispensing, *Int. J. Pharm.*, (In press 2015).

[22] N. Scoutaris, A.L. Hook, P.R. Gellert, C.J. Roberts, M.R. Alexander, D.J. Scurr, ToF-SIMS analysis of chemical heterogenities in inkjet micro-array printed drug/polymer formulations, *J. Mater. Sci: Mater. Med.*, 23 (2012) 385-391.

[23] N. Scoutaris, M.R. Alexander, P.R. Gellert, C.J. Roberts, Inkjet printing as a novel medicine formulation technique, *J. Control. Release*, 156 (2011) 179-185.

[24] A. Goyanes, A.B.M. Buanz, G.B. Hatton, S. Gaisford, A.W. Basit, 3D printing of modified-release aminosalicylate (4-ASA and 5-ASA) tablets, *Eur. J. Pharm. Biopharm.*, 89 (2015) 157-162.

[25] C.W. Rowe, W.E. Katstra, R.D. Palazzolo, B. Giritlioglu, P. Teung, M.J. Cima, Multimechanism oral dosage forms fabricated by three dimensional printing™, *J. Control.Release*, 66 (2000) 11-17.

[26] W.E. Katstra, R.D. Palazzolo, C.W. Rowe, B. Giritlioglu, P. Teung, M.J. Cima, Oral dosage forms fabricated by Three Dimensional Printing™, *J. Control. Release*, 66 (2000) 1-9.

[27] R.D. Brobyn, The human toxicology of dimethyl sulfoxide, *Ann. N. Y. Acad. Sci.* , 243 (1975) 497-506.

[28] S.W. Jacob, C. Jack, Dimethyl Sulfoxide (DMSO) in Trauma and Disease, CRC Press, Florida, 2015.

[29] H. Changqin, L. Ying, Quality Control in Pharmaceuticals: Residual Solvents Testing and Analysis, in: I. Akyar (Eds.) Wide Spectra of Quality Control, InTech, 2013, pp. 184-210.

[30] L.K. Wong, E.L. Reinertson, Clinical considerations of dimethyl sulfoxide, *Iowa State University Veterinarian*, 46 (1984) 89-95.

[31] International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Q3C (R4) Impurities: Guideline for Residual Solvents, 2009.

[32] RegenHU. (2015). 3D Discovery® instrument, 3D Bio-Printer [Photograph] Retrieved April 04 2015, from http://www.regenhu.com/products/3d-bio-printing.html

[33] P. Ige, B. Swami, T. Patil, J. Pradhan, P. Patil, P. Nerkar, S.J. Surana, Design and Development of Sustained Release Swelling Matrix Tablets of Glipizide for Type II Diabetes Mellitus Farmacia, 61 (2013) 883-901.

[34] J.L. Ford, Design and Evaluation of Hydroxypropyl Methylcellulose Matrix Tablets for Oral Controlled Release: A Historical Perspective, in: R.P. Samuel, T. Peter, D.M. Colin (Eds.) Hydrophilic Matrix Tablets for Oral Controlled Release, Springer, New York, 2014, pp. 17-51.

[35] S.A. Khaled, J.C. Burley, M.R. Alexander, J. Yang, C.J. Roberts, 3D printing of tablets containing multiple drugs with defined release profiles, *Int. J. Pharm.*, (In Press 2015).

[36] C.C. Wilson, Interesting proton behaviour in molecular structures. Variable temperature neutron diffraction and ab initio study of acetylsalicylic acid: characterising librational motions and comparing protons in different hydrogen bonding potentials, *New J. Chem.*, 26 (2002) 1733-1739.

[37] R. Esteves de Castro, J. Canotilho, R.M. Barbosa, M.R. Silva, A.M. Beja, J. Paixao, J.S. Redinha, Conformational isomorphism of organic crystals: racemic and homochiral Atenolol, *Cryst. Growth Des.*, 7 (2007) 496-500.

[38] N. Nagel, H. Schweitzer, H. Urbach, W. Heyse, B. Müller, H. Berchtold, Ramipril, *Acta Cryst. Section B*, 57 (2001) 463-465.

[39] L. Dupont, O. Dideberg, Structure cristalline de l'hydrochlorothiazide, C7H8ClN3O4S2, *Acta Cryst. Section B*, 28 (1972) 2340-2347.

[40] P.S. Collaboration, Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies, *Lancet*, 360 (2002) 1903-1913.

[41] T. Pedersen, Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S), *Atheroscler. Suppl.* , 5 (2004) 81-87.

[42] JAMA Network Webcasts, Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on Detection, Evaluation, and Treatment of high blood cholesterol in adults (Adult Treatment Panel III), *JAMA*, 285 (2001) 2486-2497.

[43] Antithrombotic Trialists' Collaboration, Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients, *BMJ*, 324 (2002) 71-86.

[44] N.J. Wald, M.R. Law, A strategy to reduce cardiovascular disease by more than 80%, *BMJ*, 326 (2003) 1419.

[45] M. Law, N. Wald, J. Morris, R. Jordan, Value of low dose combination treatment with blood pressure lowering drugs: analysis of 354 randomised trials, *BMJ*, 326 (2003) 1427.

[46] M.R. Law, N.J. Wald, A. Rudnicka, Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis, *BMJ*, 326 (2003) 1423.