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Development of a Multi-layered Vaginal Tablet Containing Dapivirine, Levonorgestrel and Acyclovir for use as a Multipurpose Prevention Technology.

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Running Title: Development of a Multi-layered Vaginal Tablet for use as a Multipurpose Prevention Technology Strategy

Keywords

Multipurpose Prevention Technology; HIV Microbicides; Contraception; Sexually Transmitted Infections; Dapivirine; Levonorgestrel; Acyclovir

Abstract

Multipurpose prevention technologies (MPTs) are preferably single dosage forms designed to simultaneously address multiple sexual and reproductive health needs, such as unintended pregnancy, HIV infection and other sexually transmitted infections (STIs). This manuscript describes the development of a range of multi-layered vaginal tablets, with both immediate and sustained release layers capable of delivering the antiretroviral drug dapivirine, the contraceptive hormone levonorgestrel, and the anti-herpes simplex virus drug acyclovir at independent release rates from a single dosage form. Depending on the design of the tablet in relation to the type (immediate or sustained release) or number of layers, the dose of each drug could be individually controlled. For example one tablet design was able to provide immediate release of all three drugs, while another tablet design was able to provide immediate release of both acyclovir and levonorgestrel, while providing sustained release of Dapivirnie for up to 8 hours. A third tablet design was able to provide immediate release of both acyclovir and levonorgestrel, a large initial burst of Dapivirine, followed by sustained release of Dapivirine for up to 8 hours. All of the tablets passed the test for friability with a percent friability of less than 1%. The hardness of all tablet designs were between 115 and 153 Newtons, while their drug content met the European Pharmacopeia 2.9.40 Uniformity of Dosage units acceptance value at level 1 and 2. Finally, the accelerated stability of all three actives was significantly enhanced in comparison to a mixed drug control.

1. Introduction

Multipurpose prevention technologies (MPTs) are products, preferably single device products, administered via a single route, that are designed to simultaneously address multiple sexual and reproductive health needs, such as unintended pregnancy, HIV infection and other sexually transmitted infections (STIs) [1]. MPTs can fall into a number of categories: 1) a drug delivery device or formulation that releases multiple active agents of which each is effective against a different indication, 2) a drug delivery device or formulation that release a single active agent that is effective against a range of different indications or 3) a barrier device such as a condom or diaphragm in combination with one or more active agents which are effective against multiple indications. According to the Coalition Advancing Multipurpose Innovations (CAMI), every minute a woman is infected with HIV, there are 86 million unplanned pregnancies around the world annually and 1 million people contract an STI every day [2]. MPTs offer a solution to these overlapping reproductive health issues using a single device, which will result in a number of benefits for the users, including convenience, increased adherence, improved effectiveness, reduction in cost and environmental impact [3].

There are currently a number of MPT products on the market, such as the male and female condom and the cervical diaphragm. The male condom is one of the key MPT barrier methods, which when used correctly is highly effective in protecting against pregnancy, HIV infection and many other common STIs [4-5]. However, inconsistent and improper use, as a result of poor acceptability, has resulted in failure rates, after one year of use, of approximately 15% and a pearl index of 15 [6], while many women cannot negotiate condom use with their partners [7]. The female

condom is a female controlled barrier method, which has been shown to have a comparable or slightly higher contraceptive efficacy when compared to the male condom [8-9] and was just as effective in reducing the recurrence of bacterial STIs [10]. Although there is no actual data on HIV prevention, mathematical modelling has suggested an effectiveness of 63 to 82% [11-12]. However, the cost of the female condom is still higher than that of the male counterpart, with strategies, such as the washing, disinfecting and reusing of the female condom being employed to try and reduce its relative cost [13]. Cervical diaphragms are designed to sit on the cervix and are traditionally used for contraception and have a similar rate of effectiveness to the male condom [14]. The cervix has a high density of CD4 cells and CCR5 chemokine receptors and has been shown to be the initial site of infection for HIV and other STIs [15-17]. Therefore, diaphragms may offer protection from HIV and other STIs and thus act as an MPT, while studies have shown that the incidences of gonorrhoea and chlamydia infections are lower in those women who use diaphragms over other barrier methods of contraception [18]. However, a large scale trial comparing the efficacy of using a diaphragm and a condom to condoms alone in preventing HIV-1, gonorrhoea and chlamydia in at-risk women demonstrated that there was no statistically significant difference between the two groups [19-20]. The Program for Appropriate Technology in Health (PATH) have developed a new ‘one size fits most’ SILCS diaphragm, which is manufactured from silicone and contains a polymer spring, rather than the metal spring used in most standard diaphragms. The SILCS diaphragm performed well in Phase I post-coital barrier effectiveness testing. However, it was recommended that for it to be most effective adjunctive use of a chemical barrier or spermicidal gel was needed [21].

PATH, has investigated the development of a SILCS diaphragm that releases the HIV microbicide dapivirine [22].

Adherence and compliance issues are well understood in contraceptive and microbicide fields, with a recent Phase IIb study of a gel containing 1%w/w of the NRTI tenofovir lowering the risk of HIV infection in sexually active women by 54% provided they reported greater than 80% adherence, which fell to 38% protection if they had between 50% and 80% adherence and to 28% with less than 50% adherence [23]. This study clearly demonstrates the influence of adherence and compliance on the efficacy of a microbicide product and suggests that any future MPT products need to consider patient adherence to the required dosing regimen, particularly for those products which are coitally dependent.

Solid dosage forms such as vaginal tablets may overcome some of the compliance, acceptability and adherence issues associated with other MPT strategies. They are more discrete, easier to administer to the vagina and are coitally independent, especially when they are designed to be sustained release tablets. A study in African women reported that 80% of the women tested liked using a vaginal tablet; while over 85% said they would definitely use them [24]. Sustained-release tablets are designed to slowly release drug at a rate governed predominately by the design of the delivery system. Many sustained release formulations are specifically designed for once-daily per-oral administration and have been shown to improve patient compliance and acceptability compared with conventional multiple daily dosing regimens [25]. Multi-layered tablets are a flexible technology that has been used for modifying the release of drugs as well as delivering multiple drugs [26-29]. Multi-layered tables have been used to achieve, zero order release [30-31], pulsatile

release [32] and even bimodal release [33], were an initial immediate release phase is followed by a period of sustained release, and then a second immediate release phase. Melt processing has be used to manufacture solid dosage forms for the purposes of solubility and bioavailability enhancement, through the productions of solid dispersions [34-37] and taste masking of bitter drugs [38-41].

This manuscript describes the development of a range of multi-layered vaginal tablets, with both immediate and sustained release layers capable of delivering the antiretroviral drug dapivirine, the contraceptive hormone levonorgestrel, and the anti-herpes simplex virus drug acyclovir at independent release rates from a single dosage form, manufactured using a combination of melt processing and compression moulding.

2. Materials and Methods

2.1 Materials

Dapivirine (DPV) was provided by the International Partnership for Microbicides, which holds exclusive rights to DPV through an agreement with Janssen Sciences Ireland UC, levonorgestrel (LNG) and acyclovir (ACY) were purchased from Cambridge Biosciences (Cambridge, UK). Sodium dodecyl sulphate (SDS) and methanol were purchased from Sigma Aldrich (Dorset, England). Kollidon® SR, Kollidon® VA Kolliphor® P 188 were provided by BASF (Ludwigshafen, Germany).

2.2 Melt rheology

Continuous flow rheological assessment of the formulations was carried out using a TA Instruments AR 2000 rotational rheometer fitted with a 40 mm diameter steel parallel plate. 3g of each formulation to be tested was placed onto the lower stationary plate of the rheometer, which was set to 200^{o}C, and the upper plate was lowered to produce a gap between the plates of 1000 μm. Excess sample was removed before initiating the test. Flow rheology was conducted in temperature sweep mode with the temperature decreased to 120^{o}C at a rate of 10^{o}C per minute.

2.3 Multi-layered tablet manufacture

The appropriate amount of polymer (Kollidon® SR or Kollidon® VA), plasticizer (Kolliphor® P 188) and active (DPV, LNG or ACY) were blended together using a laboratory sized V-blender (SOCACHIM, Brussels, Belgium) to produce a 50g powder blend. Multi-layered tablets were manufactured using combination of melt processing and compression moulding by placing the powder blend into the bottom portion of a 1mm deep mould that had been heated to either 160^{o}C (Kollidon® SR

blend) or 150^{o}C (Kollidon® VA blend) depending on the blend being processed. The blend was left in the mould for approximately 15 minutes (or until it was deemed soft enough to be compressed) and the top portion of the mould was pushed down until there was no gap between the bottom and top portions of the mould. The top portion of the mould was removed and individual layers 14mm in diameter and 1mm thick were cut from the moulded powder blend. The layers were subsequently butt welded together to form the multi-layered tablets presented in Figure 1.

2.4 Content uniformity of the individual layers

Each individual layer (n=4) was crushed using a mortar and pestle and the subsequent powder transferred to a 10mL volumetric flask. The volumetric flask was made up to volume with 10mL of 2% SDS solution and placed onto a laboratory shaker overnight at 60 RPM. A 1mL sample was removed, filtered and analysed using the dapivirine/acyclovir/levonorgestrel HPLC method. The acceptance value was calculated as per Case 1 of the European Pharmacopeia 2.9.40 Uniformity of Dosage units.

2.5 Determination of tablet hardness

The mean hardness (n = 10) of the various tablets was measured with a tablet hardness tester (Varian VK 200). Each tablet was placed in the hardness tester and the maximum force in Newton required to break each tablet was measured.

2.6 Determination of tablet friability

The friability of the tablets was determined by placing ten pre-weighed tablets, with a collective weight of approximately 3g for the three layered tablets and 4g for the four layered tablets, into the drum of a Charles Ischi AE-1 friability tester, which was

subsequently rotated 50 times at a speed of 25 RPM. At the end of the test all ten tablets were dusted off and reweighed together, the loss in the weight of tablet is the measure of friability and is calculated using equation 1. A friability of less than 1.0% is deemed a pass, if the tablets passed the first time the test was repeated using the same tablets. However, if the friability was greater than 1.0%, then the tablets failed the test and were deemed unacceptable in relation to friability.

Equation 1: Percentage Friability = W1 – W2/W1 × 100

Where: W1 = collective weight of tablets before testing

W2 = collective weight of tablets after testing.

2.7 In vitro drug release

Individual multi-layered tablets (n = 4) were placed into a sealed flask containing 10mL of 2% SDS and the flasks placed onto a laboratory shaker at 60 RPM in a walk-in incubator held at 37^{o}C. 1mL samples were taken from the release medium and filtered after 0.25, 0.5, 1, 2, 4 and 8 hours and replaced with 1mL of fresh release medium. DPV, ACY and LNG release was analysed using a dapivirine/acyclovir/levonorgestrel HPLC method.

2.8 Forced degradation stability study

Individual samples of DPV, ACY, LNG, a combination sample consisting of a 1:1:1 ratio of DPV:ACY:LNG and the various multi-layered tablet formulations were placed in an incubator in open containers and stored at 80^{o}C for 3 months to enhance degradation with samples removed after 1, 2 and 3 months. The multi-layered tablets were crushed using a mortar and pestle and transferred to a 10mL volumetric flask while the individual samples and combination samples were transferred directly

to the 10mL volumetric flask. The volumetric flask was made up to volume with 10mL of 2% SDS solution and placed onto a laboratory shaker overnight at 60 RPM. A 1mL sample was removed, filtered and analysed using the dapivirine/acyclovir/levonorgestrel HPLC method

2.9 Dapivirine/Acyclovir/Levonorgestrel HPLC Methodology

HPLC analysis was performed on a Waters Alliance HPLC with a Phenomenex Luna® C18 4.6 x 150 mm column with a 5μM particle size. A gradient flow was used as described in table 1 were mobile phase A was phosphate buffer and mobile phase B was HPLC grade acetonitrile. The flow rate was 1.00mL/min, while UV detection was performed at a wavelength of 220nm with an injection volume of 20μL. The limit of detection for the method is 0.001μg/mL while the limit of quantification is 0.01μg/mL for all 3 actives. The selectivity factor (α) between ACY and DPV was 8.1 and DPV and LNG was 1.1. The method was linear (r^2 1.00) over the standard ranges used in this study, which was 0.01 to 500μg/mL for ACY and DPV and 0.01 to 5μg/mL for LNG.

2.10 Statistical analysis

Statistical analysis was performed using a one way analysis of variance (ANOVA) (GraphPad Prism® version 5.02 for Windows, GraphPad Software, San Diego, CA). Post-hoc comparisons of the means were performed using Tukey's Honestly Significance Difference test. A significance level of $p < 0.05$ was accepted to denote significance in all cases.

3. Results and Discussion

3.1 Melt rheology

The sustained release polymer Kollidon® SR was used to provide sustained release of DPV, while the immediate release polymer Kollidon® VA 64 was used to provide immediate release of DPV, ACY and LNG. The multi-layered vaginal tablets were manufactured using melt compression moulding. Therefore, it was necessary to determine the temperatures the sustained and immediate release polymers reached their optimum melt viscosity, which is to have a sufficient decrease in viscosity to permit compression but not enough to leak from the mould during compression. This was done using melt rheology and the results presented in figure 2A and B.

The optimum melt viscosity for melt compression moulding is between 2800 and 4000Pa.S [42-43]. Figure 2A demonstrates that Kollidon® SR required a temperature of 180ºC in order to achieve an optimum melt viscosity. Therefore, we investigated the effect of a adding a plasticiser (Kolliphor® P188) on the melt viscosity of the Kollidon SR and found that increasing the amount of plasticiser decreased the temperature required to achieve the optimum melt viscosity (Figure 2A). However, the addition of too much plasticiser can have a detrimental effect on the physical characteristics (hardness, friability and drug release) of a tablet [44]. Therefore, it was decided to use 10% plasticiser and a processing temperature of 160^{o}C to manufacture the Kollidon® SR layers as this provided a melt viscosity within the optimum range (Figure 2A).

Kollidon® VA had an optimum melt viscosity at temperatures ranging from 150ºC to 180ºC, while the addition of a plasticiser had limited influence on its melt viscosity (Figure 2B) when compared to Kollidon® SR (Figure 2A). However, it was decided to use 5% plasticiser and a processing temperature of 150ºC as the addition of a small quantity of plasticiser can reduce the brittleness of the tablets and improve their hardness and friability [44].

3.2 Characterisation of the multi-layered tablets

Each layer was formulated and developed to have a specific drug content depending on the drug, type of release and the multi-layered tablet in which they were to be used. The immediate release ACY and LNG layers were formulated to contain 300 and 2μg respectively for all tablet configurations. Both the immediate and sustained release DPV layers intended to be used in the three layered tablets were formulated to contain 400μg, while those intended to be used in the four layered tablets, which contain both an immediate and sustained release DPV layer, were formulated to contain either 200 or 400μg depending on the tablet. The content uniformity data for each of the layers is presented in Table 2 and demonstrates that all of the layers contained drug contents similar to their theoretical values ($P > 0.05$). Furthermore, all of the layers meet the European Pharmacopeia 2.9.40 Uniformity of Dosage units acceptance value at level 1, accept for the 400μg immediate release DAP layer, which did meet it at level 2 (Table 2).

Major concerns with multi-layered tablets include poor mechanical properties, such as a high friability rate or low hardness, due to weaknesses at the joins between the layers [45]. However, the percent friability (Figure 4A and B), which is a method to

determine the physical strength of tablets upon exposure to mechanical shock or agitation, for all of the multi-layered tablets was less than the limit of 1.0%. Furthermore, all of the multi-layered tablets had a hardness value significantly greater ($p < 0.05$) than 40N (Figure 4C) which is deemed acceptable for vaginal tablets.

3.3 In vitro release

The multi-layered tablet technology could be used to provide an MPT strategy, were each layer would contain either a HIV microbicide, a contraceptive or a drug to reduce other STIs such as HSV, with each layer designed to provide either immediate or sustained release of any or all of the actives. In this study, a range of multi-layered tablets were developed that contained either 400 or 600μg of DPV, 300μg of ACY and 2μg of LNG. Figure 5A and B show the release profile for a multi-layered tablet that consists of three immediate release layers, one containing 400μg of DPV, one containing 300μg of ACY and the other containing 2μg of LNG. All layers released their total drug content within 1 hour, while a multi-layered tablet consisting of two immediate release outside layers (one containing 300μg of ACY and the other 2μg of LNG) and a sustained release central layer (containing 400μg of DPV) also released their ACY and LNG content within one hour while sustaining the release of DPV for up to 8 hours (Figures 5C and D).

The release profile of a multi-layered vaginal tablets consisting of three immediate release layers and a sustained release layer is presented in Figure 6. Figures 6A and B show the release profiles for tablets whose outer immediate release layers

contain 300μg of ACY and 2μg of LNG, while the central immediate release layer contains 200μg of DPV with the central sustained release layer also containing 200μg of DPV. The release profiles demonstrate that the immediate release outer layers released their ACY and LNG content within one hour, while approximately 263μg of DPV was released within an hour due to a combination of both the immediate and sustained release DPV layers, with the sustained release layer continuing to release DPV for up to 8 hours. The release profiles for a similar multi-layered vaginal tablet, only this time the central immediate release layer contains 400μg of DPV, is shown in Figures 6C and D. Like the previous tablet all of the ACY and LNG was released within the first hour. However, there is a significantly greater release ($p < 0.05$) of DPV within the first hour (477μg) due to the central immediate release layer containing twice as much DPV, while the sustained release layer continues to release DPV for up to 8 hours. These types of tablets could be used to provide an immediate 'burst' of DPV from the immediate release layer, which would provide immediate protection from HIV infection, while the sustained release layer could be used to continually 'top-up' the DPV concentration in the vaginal mucosa potentially providing protection for the rest of the day.

3.3 Enhanced degradation and stability study

The co-formulation of different active pharmaceutical ingredients into the same tablet can result in enhanced destabilisation due to incompatibilities or interactions between the actives [46]. Figure 7A demonstrates that when stored individually under conditions designed to enhance degradation all three actives maintain 100% stability after one month and after three months the ACY, DPV and LNG have

maintained 95, 93 and 90% stability respectively. However, when they are stored in combination (Figure 7B) their rate of degradation is significantly enhanced ($p < 0.05$) and within one month the ACY, DPV and LNG have only maintained 92, 90 and 85% respectively of their stability. Furthermore, after three months their stability has reduced further to 79, 76 and 75% (Figure 7B). The rate of degradation for the actives in the multi-layered vaginal tablets containing three layers (Figures 7C and D) was significantly less ($p < 0.05$) that that for the combination control (Figure 7B). After three months the stability of ACY, DPV and LNG is 93, 90 and 88% respectively for the multi-layered vaginal tablet containing three immediate release layers and 91, 94 and 86% for the tablet containing a central sustained release layer. A similar trend occurs with the multi-layered vaginal tablets containing four layers (Figures 7E and F), with the ACY and DPV having approximately 91% stability and the LNG having approximately 88% stability after three months. This is due to the drugs being formulated into separate layers, which means they do not come into contact with each other, thus the issue of incompatibilities or interactions is removed.

4. Conclusion

DPV, ACY and LNG were formulated into a range of three and four layered multi-layered vaginal tablets designed to provide independent release of each of the actives. The three layered vaginal tablets were designed to provide either immediate release of all three actives or immediate release of the ACY and LNG only, with DPV being released in a sustained fashion for up to eight hours. The four layered vaginal tablets were designed to again provide immediate release of both ACY and LNG from the two immediate release outside layers, while an immediate release central layer provided a burst of DPV within the first hour of release and a central sustained release layer provided sustained release of DPV for the next 7 hours. Furthermore, all of the tablets pass fortest for friability and met the European Pharmacopeia acceptance value for uniformity of dosage at level 1 and 2, while their hardness values were significantly greater than 40N, which is the unofficial acceptable lower limit for vaginal tablets. This platform multi-layered tablet technology has the potential for providing women with protection from both HIV and HSV infection as well as unwanted pregnancy using a single dosage form. The number of layers could be increased allowing for the delivery of more than one microbicide.

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Figure captions

Figure 1: Schematic of the various multi-layered DPV, ACY and LNG MPT vaginal tablets.

Figure 2: Melt rheology of Kollidon® SR (**A**) and Kollidon® VA (**B**) from 200 to 120ºC with varying loadings (0 to 25%) of the plasticiser Kolliphor® P188. The dashed lines are the optimum viscosity range.

Figure 3: Melt rheology of Kollidon® SR (**A**) and Kollidon® VA (**B**) from 200 to 140ºC with varying loadings (0 to 1%) of DPV and Kollidon® VA with varying loadings (0 to 1%) of ACY (**C**) and LNG (**D**). The dashed lines are the optimum viscosity range.

Figure 4: Percent friability of the multi-layered vaginal tablets containing either three (**A**) or four (**B**) layers. The average (n = 10) tablet hardness and standard deviation (**C**) for all of the multi-layered vaginal tablets.

Figure 5: The in vitro release of Dapivirine, Acyclovir (**A**) and Levonorgestrel (**B**) from a multi-layered vaginal tablet with three immediate release layers. The in vitro release of Dapivirine and Acyclovir (**C**) and Levonorgestrel (**D**) from a multi-layered vaginal tablet with two immediate release outside layers (300μg Acyclovir and 2μg Levonorgestrel) and a sustained release central layer (400μg Dapivirine).

Figure 6: The in vitro release of Dapivirine, Acyclovir (**A**) and Levonorgestrel (**B**) from a multi-layered vaginal tablet with two immediate release outside layers (300μg Acyclovir and 2μg Levonorgestrel) an immediate release central layer (200μg Dapivirine) and a sustained release central layer (200μg Dapivirine). The in vitro release of Dapivirine, Acyclovir (**C**) and Levonorgestrel (**D**) from a multi-layered vaginal tablet with two immediate release outside layers (300μg Acyclovir and 2μg Levonorgestrel) an immediate release central layer (400μg DPV) and a sustained release central layer (200μg Dapivirine).

Figure 7: Percent stability of DPV, ACY and LNG stored at 80°C in open containers in the individual control (**A**), combination control (**B**), multi-layered vaginal tablets with 3 layers (**C** and **D**) and multi-layered vaginal tablets with four layers (**E** and **F**).

Table captions

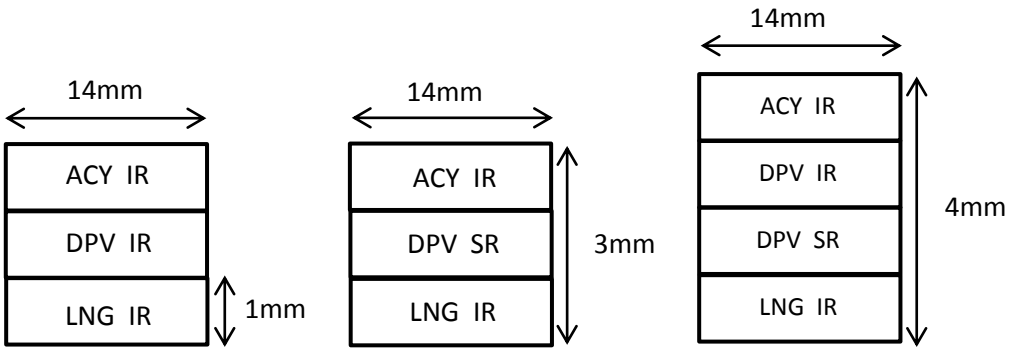
Table1: Mobile phase composition for the gradient HPLC method

Table 2: Theoretical and actual drug content for each of the individual tablet layers.

Time (Min)	Mobile Phase A (%)	Mobile Phase B (%)
0	40	60
3	40	60
10	30	70
15	30	70
25	40	60

Drug	Dapivirine				Acyclovir	Levonorgestrel
Release	Immediate		Sustained		Immediate	Immediate
Theoretical (μg)	400	200	400	200	300	2
Actual (μg) (*Std Dev*)	403.2 (*6.3*)	201.9 (*2.9*)	402.7 (*5.7*)	202.1 (*3.1*)	301.9 (*2.5*)	2.1 (*0.19*)
Acceptance Value	15.4	7.0	13.7	7.4	6.0	5.5

Figure 1



DPV IR = Dapivirine Immediate Release

DPV SR = Dapivirine Sustained Release

ACY IR = Acyclovir Immediate Release

LNG IR = Levonorgestrel Immediate Release

Figure 2

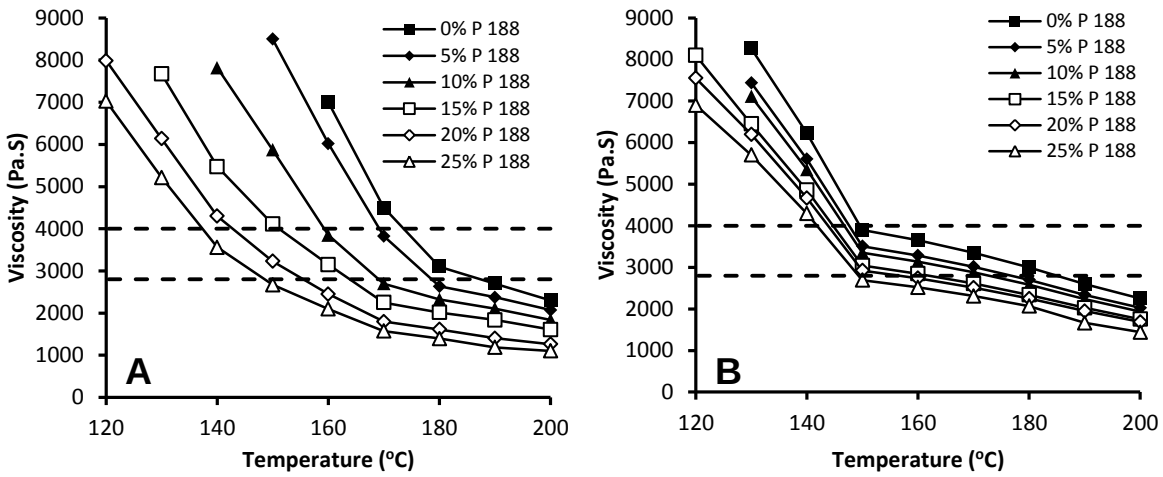


Figure 3

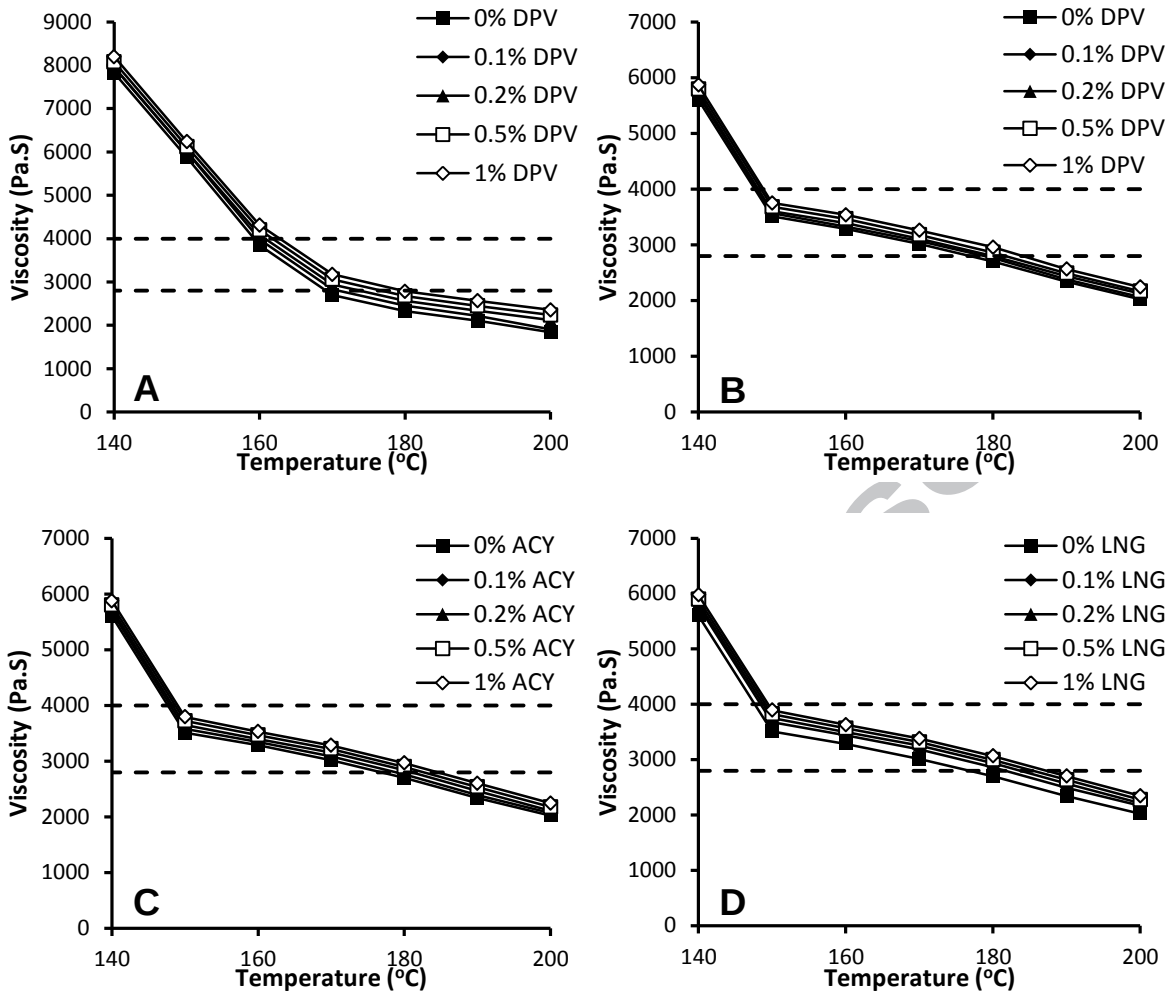


Figure 4

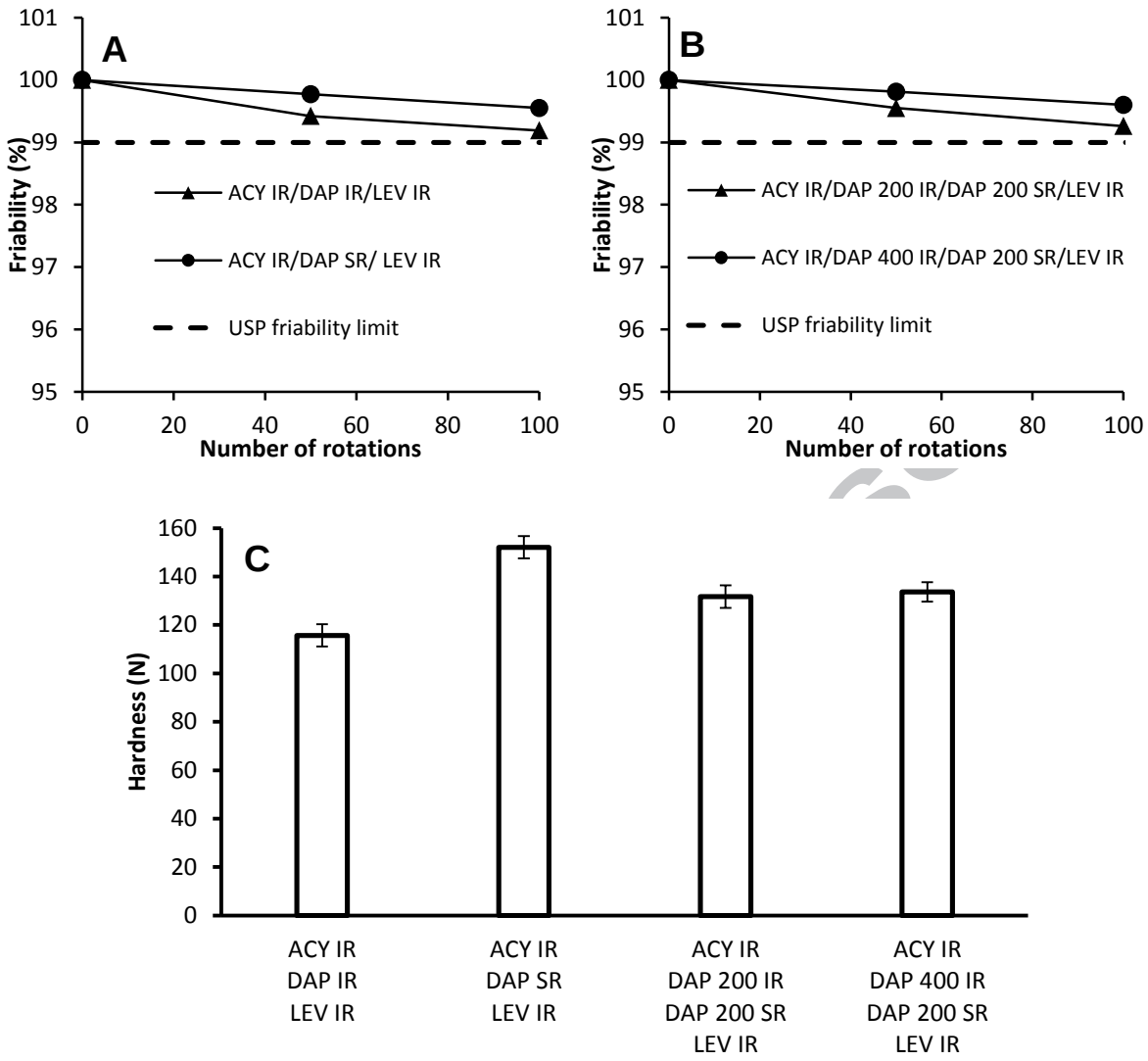


Figure 5

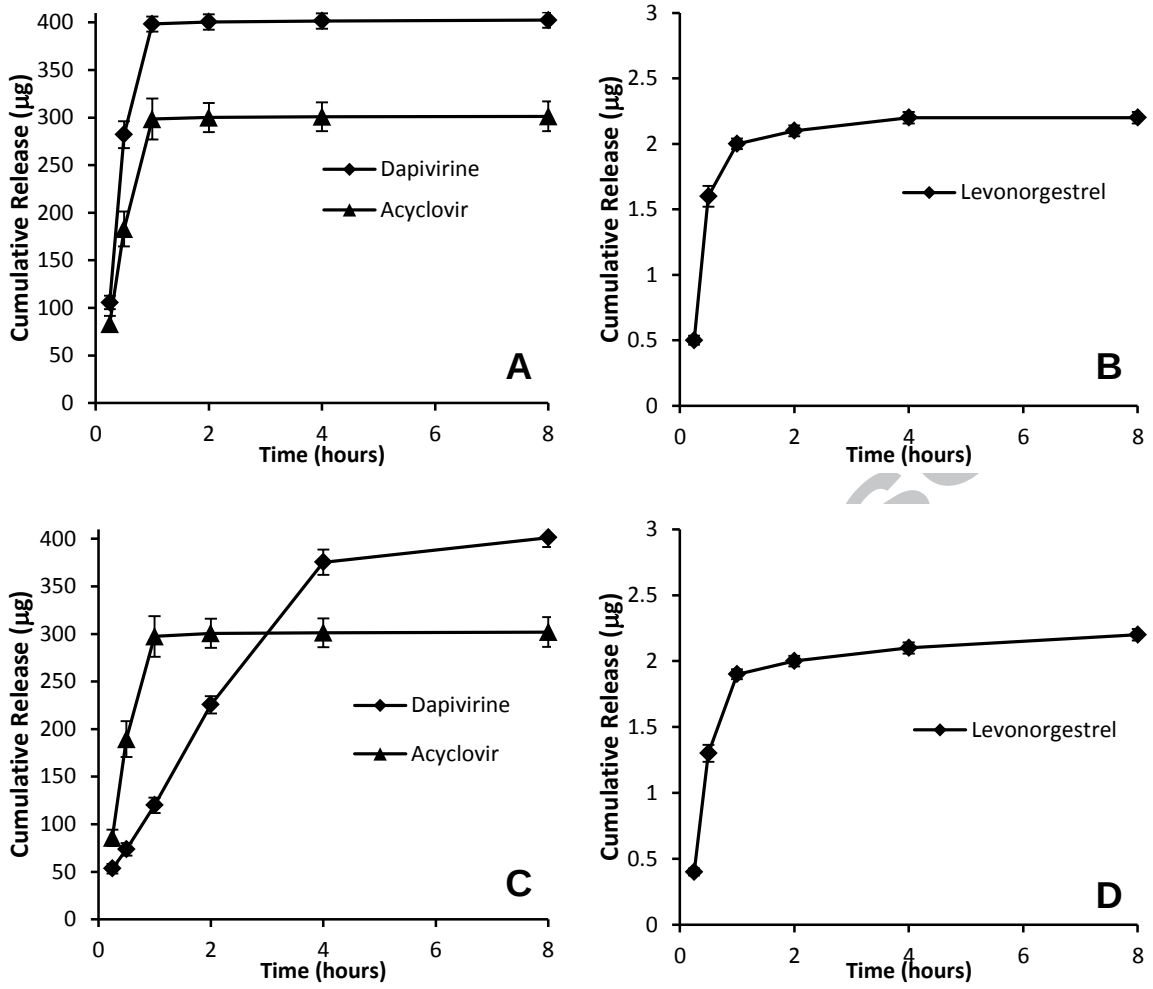


Figure 6

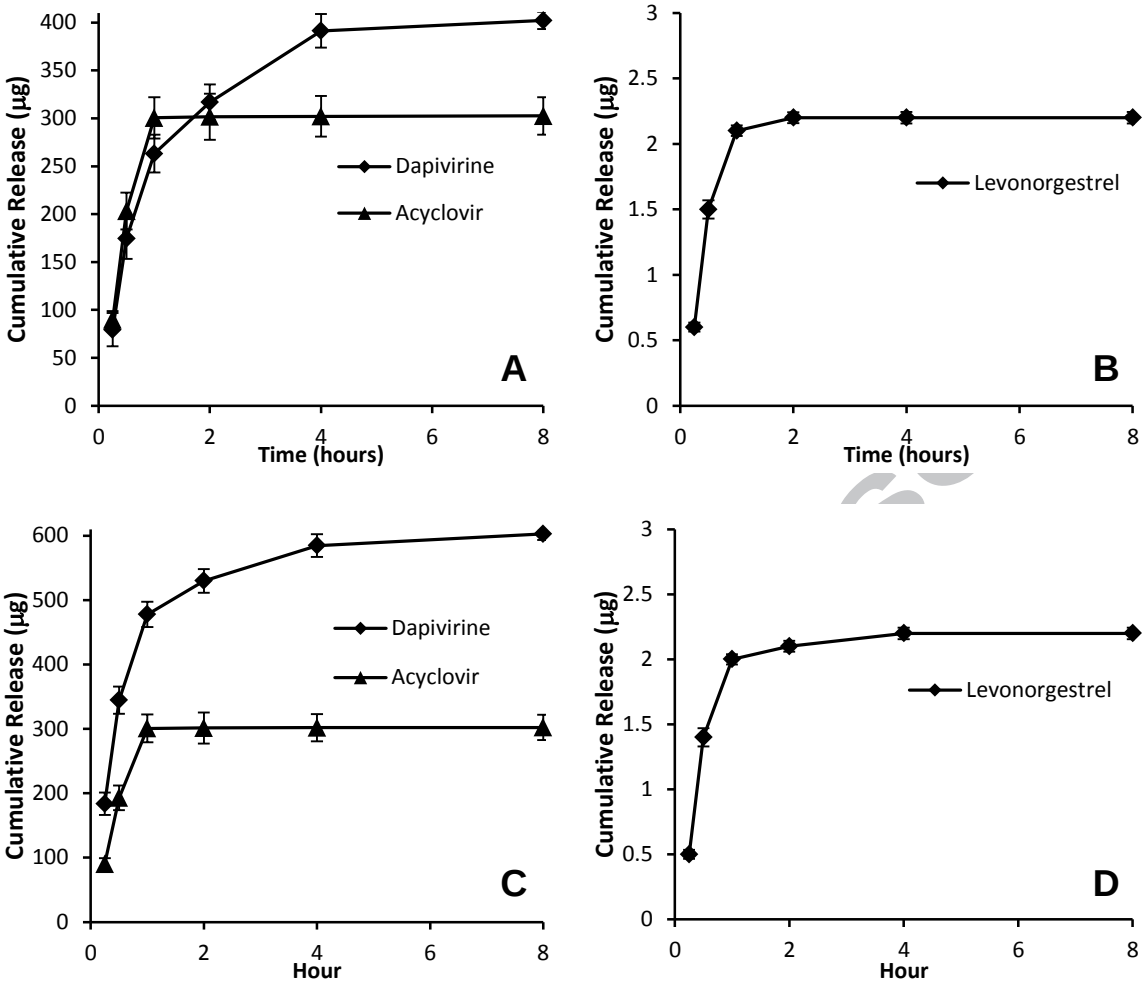
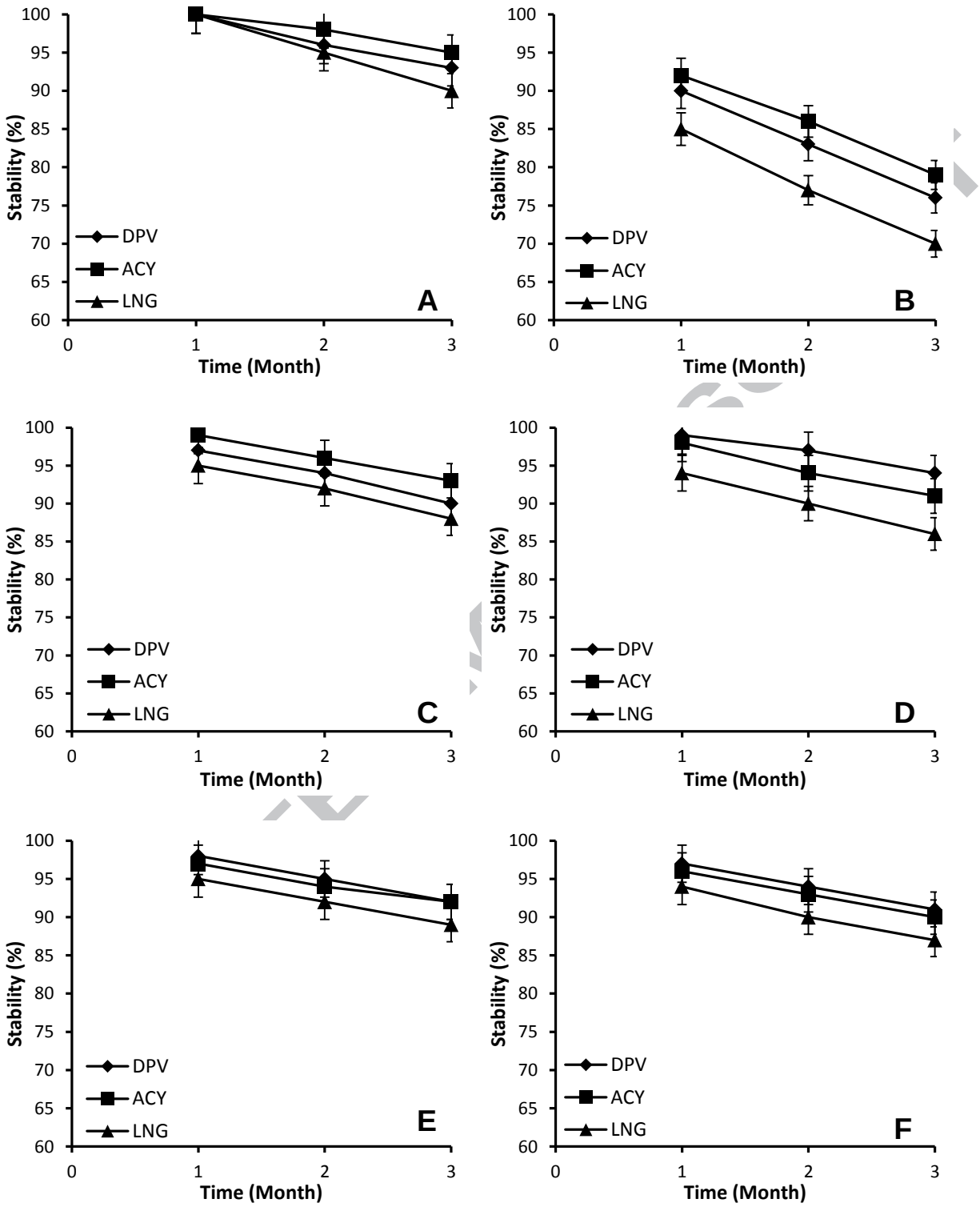


Figure 7



Graphical Abstract