Disulfiram-loaded immediate and extended release vaginal tablets for the localised treatment of cervical cancer

Development of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets for the Localised Treatment of Cervical Cancer

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Keywords

Disulfiram, Cervical Cancer, Vaginal tablet, Localised Delivery, Immediate and Sustained Release

Abstract

Objectives To develop and manufacture both immediate and sustained release vaginal tablets containing the anticancer drug disulfiram, which has the potential to be used as a non-invasive treatment for cervical cancer.

Methods Disulfiram-loaded vaginal tablets were manufactured at pilot scale using the direct compression method. These tablets were tested in accordance with the European Pharmacopeia testing of solid dosage form guidelines. They were also tested using a bio relevant dissolution method as well as a dual-chambered release model designed to better mimic the dynamic nature of the vaginal vault.

Key findings We have developed both immediate and sustained release vaginal tablets, which when manufactured at pilot scale are within the limits set by the European Pharmacopeia for the testing of solid dosage forms. Furthermore, these tablets are capable of releasing disulfiram *in vitro* using the dual-chambered release model at levels 25 000 times and 35 000 times greater than its IC50 concentration for the HeLa cervical cancer cell line.

Conclusions The successful pilot manufacture and testing of both the immediate and sustained release disulfiram-loaded vaginal tablets warrant further investigation, using an in-vivo model, to assess their potential for use as a non-invasive treatment option for cervical cancer.

1. Introduction

Cervical cancer is officially the third most prevalent cancer in women globally, with 529,000 cases a year, 275,000 of which result in death. It is especially dominant in developing countries where approximately 85 per cent of cases arise due to the lack of cervical cancer prevention and control programs. In developed countries, where women have access to resources capable of detecting and treating precancerous lesions, the number of cases is reduced by approximately 80% [1]. The main cause of cervical cancer is the sexual transmission of the human papillomavirus (HPV). Approximately 15 HPV types are classed as carcinogenic or high-risk. However types 16 and 18 are the most carcinogenic and are the main contributors to cervical cancer [2]. HPVs are small double-stranded DNA viruses that have the ability to infect epithelial cells in the cervix after entering a host through sexual contact [3]. The infected individual will produce antibodies as an immune response to the infection [3]; therefore most HPV infections are temporary and will be resolved within 6 to 12 months. However, persistent HPV infections have the potential of causing the development of precancerous lesions and cervical intraepithelial neoplasia (CIN) that may lead to cervical cancer [4]. Many other risk factors, such as sexual activity at a young age, multiple sex partners, multiple pregnancies, smoking, oral contraceptives and other sexually transmitted infections can also contribute to cervical cancer [2-3].

In carcinoma in situ (stage 0), abnormal cells are found in the innermost lining of the cervix. These abnormal cells may become cancerous and spread into nearby normal tissue. Stage I cancer is found in the cervix only and can be divided into stages IA and IB depending on the amount of cancer found. These can be further divided into stages IA1, IA2, IB1 and IB2 depending on the size of the tumour. In stage II the

cancer has spread beyond the cervix but not as far as the pelvic wall or the lower third of the vagina. Stage II is also divided into IIA and IIB, based on how far the cancer has spread. In stage III the cancer has spread to the lower third of the vagina and/or pelvic wall and may also have started to cause kidney problems. Like stage II, stage III is also divided into stages IIIA and IIIB, based on how far the cancer has spread. By stage IV the cancer has spread to bladder, rectum or other parts of the body. Stage IV can also be divided into IVA and IVB, depending on where the cancer is found. In stage IVA the cancer has spread to nearby organs such as the bladder and rectum, while in stage IVB, the cancer may be found in other parts of the body, such as the liver, lungs, bones or distant lymph nodes.

In developed countries, there are currently two methods used to prevent cervical cancer from developing to an advanced stage: (1) the distribution of HPV vaccines that are mainly directed against HPV types 16 and 18 [5] and (2) screening methods, such as Papanicolaou test (Pap smear), which involves the collection of exfoliated cells from the cervix, which are then examined for cellular abnormalities. This enables identification of CIN before they begin to develop into cervical cancer [6].

Following the diagnosis of cervical cancer there are many different treatment options and methods available, which depend on the stage of the cancer and whether or not the woman would like the option to be able to have children after treatment. Treatments for pre-invasive cancer (stage 0) include loop electrosurgical excision procedure (LEEP), large loop excision of the transformation zone (LLETZ) or cone-shaped excision and involve the removal of a small amount of cervical stroma [7]. Microinvasive cancer (stage IA) is treated by a hysterectomy (removal of the whole

uterus including part of the vagina), while in stage IA2 cancers, the lymph nodes are removed as well. Early stage cancers (1B1 and IIA1) can be treated with a radical hysterectomy and removal of the lymph nodes or using radiation therapy, which may be given using external beam radiotherapy to the pelvis and/or brachytherapy via the vagina. Patients treated using surgery may also receive radiation therapy in order to reduce the risk of relapse. Larger early stage cancers (IB2 and IIA2) are treated with either radiation therapy or chemotherapy or a hysterectomy followed by radiation therapy, or chemotherapy followed by a hysterectomy. Advanced stage cancers (IIIA to IVB) are treated using radiation therapy and chemotherapy [8]. All of these treatment options are either very invasive or involve extended stays in or repeated visits to the hospital. The location of the cervix makes it easily accessible through the vagina and allows for non-invasive localised delivery of chemotherapeutic drugs, which offers a number of advantages over systemic administration, which include increased drug stability as it remains in the delivery device until released, direct delivery to the site of action, lower dose of drug required and reduced side effects due to the avoidance of systemic circulation [9]. Furthermore, cervical cancer is an excellent choice for localised drug delivery as due to regular screening approximately 50% of cases are diagnosed when the cancer is confined to the cervix (localised; stage I), while about 35% of cases are diagnosed after the cancer has spread to the lymph nodes (regional; stage II/III). Therefore, only about 10% of cases are diagnosed when the cancer has already spread to distant regions (metastasized; stage IV).

The vagina has been used to deliver drugs for a range of clinical and research applications, including contraception, vaginal infections and HIV prevention, with

many different vaginal formulations such as gels, creams, pessaries, suppositories rings, films and tablets available [10-18]. However, only a small number of these delivery systems have been investigated for the localised delivery of chemotherapeutic drugs to the cervix [19-23]. The vaginal tablet is a very common dosage that are easy to manufacture, easy to place in the vagina and have a low cost of manufacture, making them ideal for use in developing countries. Conventional tablets for vaginal application may contain binders, disintegrants, and other excipients commonly used in tablets intended for oral administration. As with most tablets the release rate can be varied by changes in the formulation or manufacturing process (i.e. the compression of the tablets). Mucoadhesive polymers can be added to the formulation to help improve their retention time in the vagina. Disulfiram is currently used for treating alcohol abuse as it can inhibit the enzyme aldehyde dehydrogenase that is responsible for metabolizing alcohol. However, disulfiram has shown potential anti-tumour activity as it can induce apoptosis in some cell lines and reduce cell growth in certain tumours [24]. Disulfiram is also capable of inhibiting activity of NFκB, a chemoresistant anti-apoptotic factor found in many cancers [25]. An anticancer effect has been demonstrated in prostate cancer, breast cancer, lung cancer, leukaemia and cervical adenocarcinoma [25-32].

In this study we show, for the first time, the development and characterisation of both immediate and extended release vaginal tablets that are capable of releasing the chemotherapeutic drug disulfiram at levels well above its IC50 value for the HeLa cervical cancer cell line.

2 Materials and Methods

2.1 Materials

Microcrystalline cellulose was purchased from Alfa Aesar (Lancashire, UK), Maize starch was obtained from Fisher Scientific (New Jersey, USA) and Lactose (α-D Lactose Monohydrate) was obtained from Acros Organics (Antwerp, Belgium). Methylcellulose, Kollidon® SR, Magnesium stearate, Stearic acid, Sodium Dodecyl Sulphate (SDS) and Disulfiram were purchased from Sigma-Aldrich, (Dorset, UK). All were used as supplied

2.2 Determination of the IC_{50} value for Disulfiram on the HeLa Cervical Cancer Cell Line

The HeLa cervical cancer cells were exposed to different concentrations of Disulfiram (n = 3) in combination with 10 μM of CuCl2 (10uM) for 72h and the concentration required to kill 50% of the cells was determined using the MTT cell proliferation assay.

2.3 Determination of flowability, bulk and tapped density of the pre-compression active mixes

The active mixes where prepared in accordance with table 1 and the excipients where mixed together in a Speed Mixer DAC 15FVZ-K (Synergy Devices Ltd.) for 5 min at 3500 RPM. 25 grams of each active mix was placed into the measuring cylinder of a Varian USP single-platform tapped density analyser. The bulk density was calculated by dividing the weight (g) by the volume (mL). The cylinder was then tapped and the volume measured at intervals of 10 taps and the tapped volume determined when there was no change in volume after 3 consecutive readings [33].

The tapped density was calculated by dividing the weight (g) by the tapped volume (mL). The Carr's index and Hausner ratio (calculated using equations 1 and 2, respectively) were used to determine the flowability of the active mixes.

Equation 1: Carr's index = 100 X (1 – B/T)

Equation 2: Hausner ratio = T/B

Where B is the bulk density of the active mix and T is the tapped density of the active mix

The powder flow of the active mixes was evaluated using an Erweka GTL flowability tester, where 25 grams of each active mix was added to a dry funnel with the opening blocked. The opening was then opened and the rate taken for the sample to flow out of the funnel was measured [34].

2.4 Manufacture of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets

The active mixes where prepared in accordance with table 1 and the excipients where mixed together in a Speed Mixer DAC 15FVZ-K (Synergy Devices Ltd.) for 5 min at 3500 RPM. The active mixes where added to the hopper and compressed on an eight punch pilot scale tableting press (STC Rotary) to produce tablets of approximately 400mg.

2.5 Determination of the hardness of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets

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 [35].

2.6 Determination of the friability of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets

The friability of the Disulfiram-loaded immediate and extended release vaginal tablets was determined by placing ten pre weighed tablets with a collective weight of approximately 4 grams 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 the tablets were dusted off and reweighed, the loss in the weight of tablet is the measure of friability and is calculated using equation 3. 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 [36].

2.7 Uniformity of weight of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets

Twenty randomly selected immediate and extended release tablets were individually weighed and their average weight determined [37].

2.8 Content Uniformity of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets

Ten individual tablets were crushed and the powder was dissolved in 1000mL of 2% SDS solution and filtered using a 0.45 micron filter before being analysed using the Disulfiram HPLC method [38].

2.9 Determination of the disintegration time of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets

One tablet was place into each of six tubes (held in place with a disk) on the basket of a Varian VK 100 single basket tablet disintegration tester, before being suspended in a beaker containing 900mL of deionised water and the time taken for all tablets to disintegrate was recorded [39].

2.10 Dissolution of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets

One pre-weighed tablet was placed into each of six vessels on a Varian 705 DS dissolution apparatus, which contained 900mL of 2% SDS solution heated to 37^{o}C. The paddle speed was set at 100 RPM and 5mL samples were withdrawn (and replaced with pre-heated dissolution media) at 15 minute intervals and analysed using the Disulfiram HPLC method until approximately 100% of the total drug content was released [40].

2.11 Biorelevant Dissolution of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets

A biorelevant dissolution study was performed in order to resemble the aqueous environment and limited fluid levels of the vagina. The tablets (n = 4) were placed into 8mL of deionised water and placed into an orbital shaking incubator (Innova 43)

at 37^{o}C and 60 rpm. The release medium was sampled after 15, 30, 45, 60, 90, 120, 240, 480 and 1440 minutes with complete replacement of the release medium and analysed using the Disulfiram HPLC method.

2.12 In vitro release of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets using a dual chambered release method

Two further mimic the dynamic nature of the vagina, were you have an aqueous environment (vaginal vault) surrounded by hydrophobic tissue, each Disulfiram-loaded tablet (n = 4) was placed into a sealed latex balloon, with a 5 mm thick wall (to simulate vaginal tissue), containing 8 mL of water to simulate vaginal fluid. The balloons where then submerged in 100 mL of 2% SDS solution (to simulate tissue) and placed into an orbital shaking incubator (Innova 43) at 37^{o}C and 60 rpm. 2mL mL of sample was taken from the water and 5mL from the 2% SDS solution at 15, 30, 45, 60, 90, 120, 240, 480 and 1440 minutes and replaced with fresh media. The samples were analysed using the Disulfiram HPLC method.

2.13 Disulfiram HPLC Methodology

HPLC analysis was performed on an Agilent 1200 series HPLC with a Phenomenex Luna C18 4.6 x 150 mm column with a 5 μM particle size. The mobile phase was comprised of 80% HPLC grade methanol and 20% HPLC grade water. The flow rate was 1.00mL/min, while UV detection was performed at a wavelength of 275nm with an injection volume of 10μL.

2.14 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 Determination of the IC_{50} Value of Disulfiram on the HeLa Cervical Cell Line

Figure 1 demonstrates that disufiram has a dose-dependent inhibition of the HeLa cervical cancer cell line with an IC_{50} value of 124.3nM (*std dev 3.6*). Furthermore, it also demonstrates that disulfiram is much more potent against the HeLa cervical cancer cell line than it is against various breast cancer, glioblastoma and prostate cancer cell lines [25-29]. Disulfiram has been already been tested in both preclinical and clinical studies for its safety and effectiveness against a range of cancers, with promising results [30-32]. Therefore, due to its greater potency in cervical cancer cells, compared to other cell lines, it would make sense to develop a formulation capable of the localised delivery of disulfiram to the cervix for the treatment of cervical cancer and consequently we have developed both immediate and extended release disulfiram-loaded vaginal tablets.

3.2 Flowability, bulk and tapped density of the pre-compression active mixes

The successful manufacture of a tablet formulation is dependent on the flow characteristics of the powdered formulation [41]. The flow of a powder is important in determining its compression characteristics and poor powder flow can result in the tablets having varying weights, content uniformity, hardness, friability and dissolution rates [42]. Therefore, it is important to characterise and optimise powder flow in the manufacture of tablets. There are a number of methods commonly used to test powder flow; some examples include Carr's compressibility index, Hausner ratio, angle of repose and flow-through-an-orifice [34, 43-45]. In this study we choose to

use Carr's compressibility index, the Hausner ratio and flow-through-an-orifice. Figure 2A demonstrates that the extended release active powder mix required less taps to become compacted and that the per cent volume change was much smaller when compared to the immediate release active mix (P values < 0.05). Furthermore, the extended release active mix had a Carr's index and Hausner ratio of 9.98 and 1.03 respectively, which would suggest that it had excellent flow properties, while the Carrs's index and Hausner ratio for the immediate release active where 14.87 and 1.16 respectively giving it good flow properties (Figure 2B). These results demonstrate that both active mixes are free flowing due to weak inter-particle forces and thus would be easily compressed on a tablet press. This is further evident in figure 2C, which shows that both active mixes had average flow rates of 37.5 and 27.5 g/s respectively, with standard deviations, after five readings, of zero, indicating an even and consistent flow of powder through the orifice of the funnel, which is within the specification limits of the European Pharmacopeia flowability monograph [34]. The reason for the extended release active mixes having a significantly higher flow rate (P values < 0.05) is due to the fact that it contains 37% of the excipient Kollidon® SR, which is a hydrophobic polymer used as a matrix in both extended and sustained release formulations. Kollidon® SR has been shown to have excellent flow properties and can also enhance the flowability of other excipients in a tablet formulation [14].

3.3 Characterisation of Disulfiram-Loaded Vaginal Tablets

3.3.1 Uniformity of Weight and Content Uniformity

It is important that the tablets have uniformity of weight as this will provide an indication that the drug content within each tablet is also uniform. Figure 3A

demonstrates that both the immediate and extended release vaginal tablets comply with the European Pharmacopeia 'uniformity of mass of a single-dose preparation' monograph [37] as none of the individual tablets deviated from the average (421.7 and 436.5) by more than 5%. The difference in the average weight of the two tablet types (P values < 0.05) is due to the fact that the extended release active mix has better flow characteristics compared to the immediate release active mix (Figure 2B and C) and thus requires less agitation to become completely compacted (Figure 2A). Therefore, more of the extended release active mix will enter the die of the tablet press, which will result in the tablets having a greater mass.

Based on the average weight of the tablets and drug loading of 30% the theoretical disulfiram content of both the immediate and extended release vaginal tablets should be 126.5 and 131.0 respectively. Figure 3B demonstrates that the both the immediate and extended release tablets had an average drug content of 124.5 and 133.0mg respectively. Furthermore, both sets of tablets comply with the European Pharmacopeia Uniformity of Content of Single-Dose Preparations monograph as none of the 10 tablets tested were outside the specification limits of 85 to 115% of the theoretical content.

3.3.2 Tablet Hardness and Friability

Figure 3C demonstrates that the extended release tablets have a significantly higher hardness compared to the immediate release tablets (P values < 0.05), with values of 84.7 and 54.9N respectively. The reason for this is that the extended release active mix has significantly higher flowability compared to the immediate release active mix (Figure 2C), which results in a higher compressibility index (Figure 2B),

resulting in much harder tablets. Furthermore, as has been mentioned previously, the extended release tablets contain 37% of the excipient Kollidon® SR, which has excellent flowability, compressibility and binding properties and thus will significantly contribute to the hardness of the tablet.

The percent friability (Figure 3D), which is a method to determine the physical strength of tablets upon exposure to mechanical shock or agitation, was greater for the immediate release tablets compared to the extended release tablets. This is because friability is related to tablet hardness - the harder the tablet the less friable it will be. According to the European Pharmacopeia Friability of Uncoated Tablets monograph a percent friability of less than 1.0% is acceptable [36]. Therefore, both the immediate and extended release tablets are within the monograph's specifications.

3.3.3 Tablet Disintegration and Dissolution

The disintegration of tablets is an important step in the dissolution process and is when the tablet breaks up into much smaller particles allowing for the increased release of drug. According to the European Pharmacopeia Disintegration of Tablets and Capsules monograph, immediate release tablets must disintegrate within 15 minutes, there is no requirement to test the disintegration rate of extended release tablets [39]. However, we tested our extended release tablets to see if they took significantly longer than the immediate release tablets to disintegrate, thus ensuring the extended release of disulfiram. Figure 3E demonstrates that the immediate release tablets disintegrated within 13 minutes and thus where within specification, while the extended release tablets took 243 minutes to disintegrate, which is

significantly longer than the immediate release tablets (P values < 0.05) and should result in the prolonged release of disulfiram.

Figure 3F shows the percentage release of disulfiram from both the immediate and extended release vaginal tablets under sink conditions. The immediate release tablet released approximately 100% of its disulfiram content within the first two hours, while it took up to four hours for the extended release tablet. Looking at the extended release profile it would suggest that the drug is released at a zero-order release rate. However, using the Korsmeyer–Peppas equation or power law, it was shown that the extended release tablets had an exponent *n* value of 1.23 (r^2 0.99) which would suggest that the tablets exhibited Case-II transport release kinetics, in which the dominant mechanism for drug release is due to polymer relaxation as the Kollidon® (37% loading) swells. This can be demonstrated by the change in thickness of the tablets from an average diameter of 12mm and a width of 4mm before release, which increased to 15 and 5.2mm respectively after release. This was an increase of approximately 23% in diameter and 30% in width. Furthermore, lactose (30% loading), due to its water soluble and hydrophilic nature, has been shown to enhance the swelling characteristics of tablets and is considered to be one of the better excipients at achieving controlled drug release from tablets manufactured using natural and/or synthetic controlled release polymers [46]. The immediate release tablets also exhibited Case-II transport release kinetics, particularly over the early stages of release, with an exponent n value of 1.67 (r^2 0.99). This again is due to its high loading (27.9%) of lactose as well as methyl cellulose (25%), which is also known to cause tablets to swell.

3.5 Biorelevant Dissolution of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets

In order to replicate the aqueous environment and limited vaginal fluid levels of the vagina, as well as the constant replacement of vaginal fluid we placed the tablets into 8mL of water, with constant replacement of the release medium. Figure 4A demonstrates that the immediate release tablets can achieve a disulfiram concentration (68.4 to 103.7 mM) greater than its IC50 value of 0.125mM. However, after 8 hours of release the concentration to fall to below the IC50 value by 24 hours. The reason for the aforementioned release profile is because the immediate release tablets begin to disintegrate straight away(Figure 5A to E) releasing the disulfiram. However, after 8 hours the tablets have completely disintegrated (Figure 5F) and the debris from the tablet is removed from the release vessel upon replacement of the release media. Therefore, the amount of drug available for release is decreased each time the release media is replaced. This scenario would also happen *in vivo* as women produce about 6g of vaginal fluid per day, with about 0.5 to 0.75g present at any one time [47]. However, in figure 4A the extended release tablets release disulfiram at levels (67.5 to 630.4 mM) above the IC50 up to 24 hours when the release was stopped. The reason for this is that the extended release tablets demonstrate minimal disintegration across the 24 hours of release (Figures 5G-M) and therefore minimal amounts of the tablets are removed upon replacement of the release media. Therefore, *in vivo*, the sustained release tablet should remain within the vagina and continue to release drug onto the cervix for length of time they are kept in place.

The cumulative release data (Figure 4B) demonstrates that both the immediate and extended release tablets can achieve disulfiram concentrations well in access of the IC50 concentration after 24 hours of release, 814.6 and 1189.5 mM respectively. However, this only correlates to approximately 1.5 and 2.2% respectively of their total disulfiram content. This is due to the low aqueous solubility of disulfiram which has a log P value of approximately 3.88.

The vagina is a much more dynamic environment compared to the single compartment release study described above. The vaginal vault may contain aqueous vaginal fluid, but it is a collapsed space surrounded by vaginal tissue which is much more hydrophobic. Therefore, to represent the two compartments (aqueous vaginal vault surrounded by hydrophobic tissue) we used a more dynamic dual chambered release model consisting of a balloon filled with water to represent the aqueous vaginal vault, which was submerged in 100mL of 2% SDS solution to represent the hydrophobic tissue.

3.6 In vitro release of Disulfiram-Loaded Immediate and Extended Release Vaginal Tablets using a dual chambered release model

Figure 6A shows the release profile of disulfiram from both the immediate and extended release tablets into the balloon, which represents the aqueous vaginal vault, to be similar to their release profiles in figure 4A. This is to be expected as the release rate of disulfiram is being controlled by its solubility in the aqueous media. However, figure 6B demonstrates that a much greater concentration of disulfiram is diffusing across the balloon wall (which represents the vaginal wall) and into the 2% SDS solution (which represents tissue absorption). This dual chambered model

demonstrates that both the immediate and extended release vaginal tablets can achieve tissue concentrations (3182.8 and 4410.8 mM respectively) well in access of the disulfiram IC50 for cervical cancers cells.

4. Conclusion

This paper demonstrates the development of both immediate and extended release vaginal tablets that are capable of delivering the chemotherapeutic drug disulfiram at levels significantly greater than its IC50 value for the HeLa cervical cancer cell ine. Furthermore, the powder formulations have both good and excellent flow properties, respectively, which allows them to be mass produced on an eight punch pilot scale tablet press and the subsequent tablets pass all European Pharmacopeia tests, with respect to hardness, friability, content uniformity, uniformity of weight, disintegration and dissolution. We believe that these tablet formulations offer a non-invasive alternative to the current cervical cancer treatments available and the localised delivery should provide a better bioavailability of drug at the cancer site, while also minimising the systemic side effects associated with chemotherapeutic drugs.

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Figure 1:

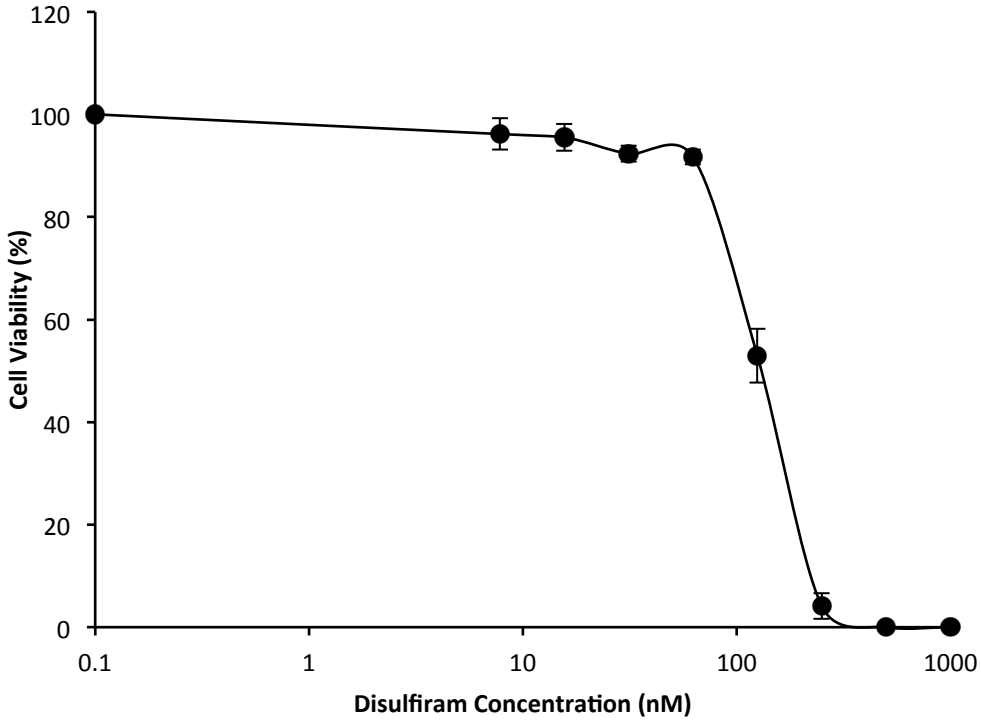
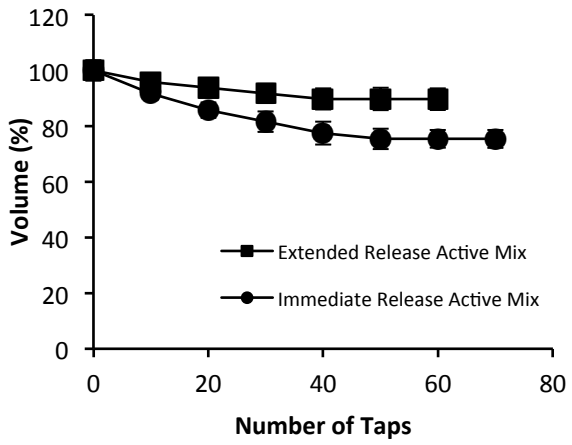
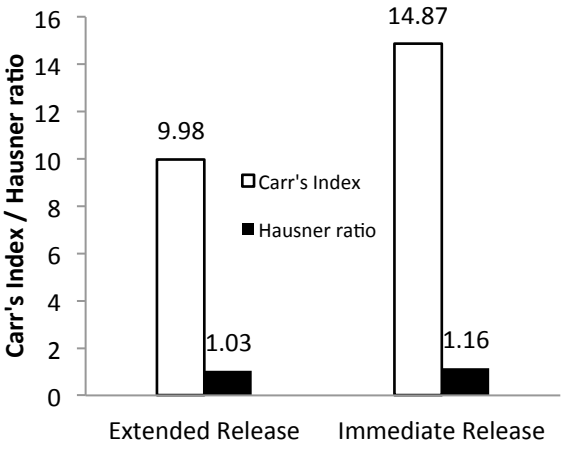

120
100
80
60
40
20
0
Cell Viability (%)
0.1
1
10
100
1000
Disulfiram Concentration (nM)

Figure 2:


120
100
80
60
40
20
0
Volume (%)
0
20
40
60
80
Number of Taps
Extended Release Active Mix
Immediate Release Active Mix


16
14
12
10
8
6
4
2
0
Carr's Index / Hausner ratio
9.98
14.87
1.03
1.16
Carr's Index
Hausner ratio
Extended Release
Immediate Release

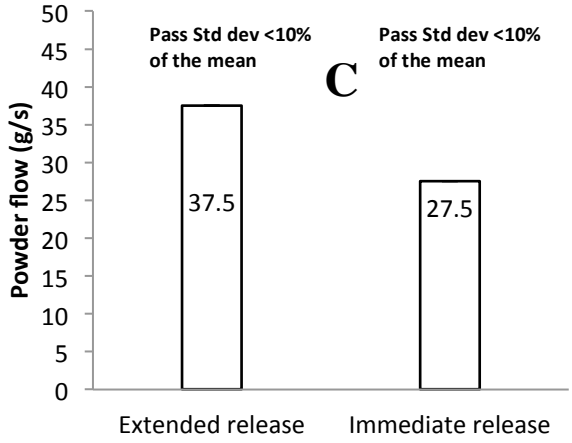

50
45
40
35
30
25
20
15
10
5
0
Powder flow (g/s)
Pass Std dev <10% of the mean
Pass Std dev <10% of the mean
C
37.5
27.5
Extended release
Immediate release

Figure 3:

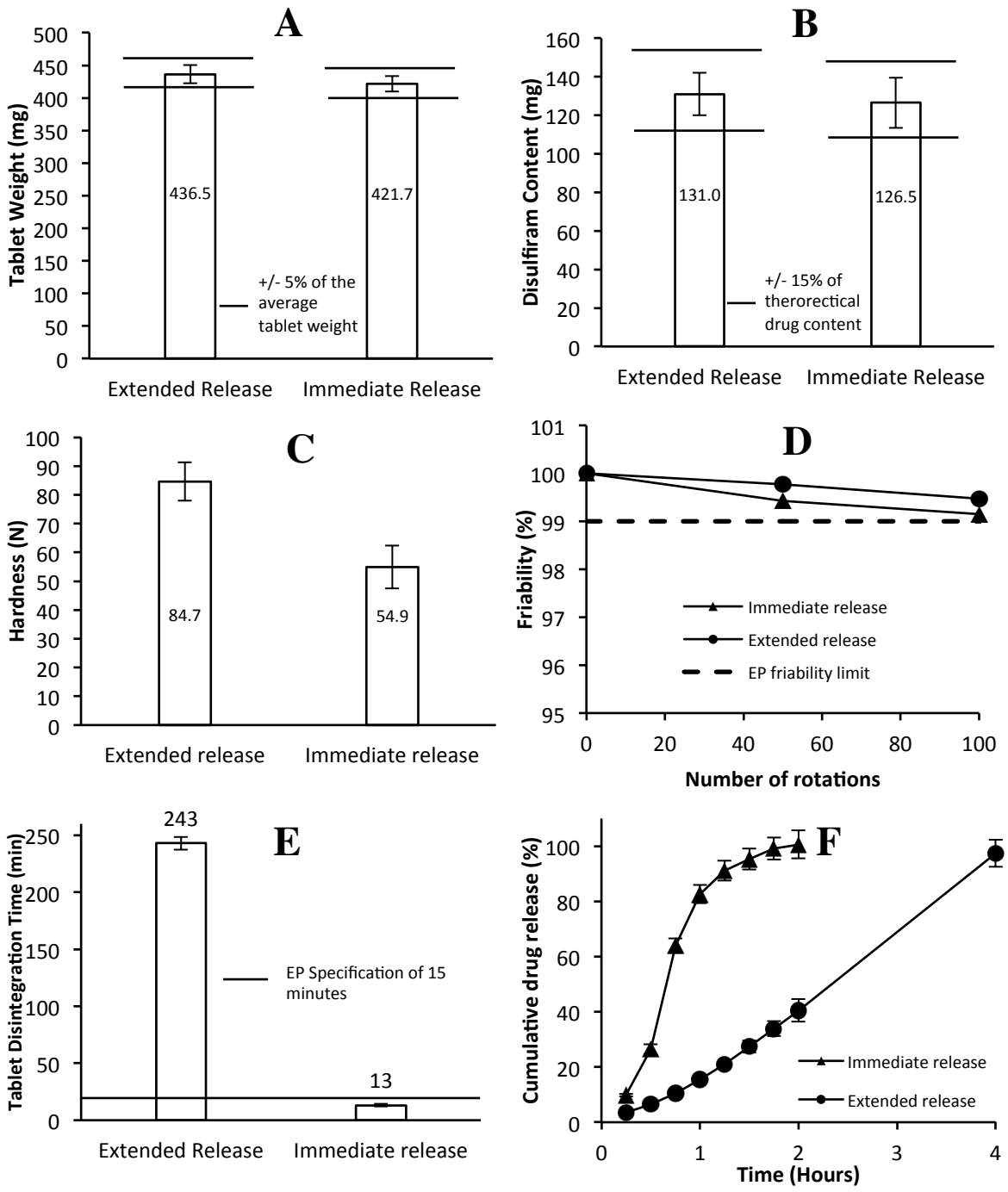

A
Tablet Weight (mg)
500
450
400
350
300
250
200
150
100
50
0
436.5
421.7
+/- 5% of the average tablet weight
Extended Release
Immediate Release
B
Disulfiram Content (mg)
160
140
120
100
80
60
40
20
0
131.0
126.5
+/- 15% of therorectical drug content
Extended Release
Immediate Release
C
Hardness (N)
100
90
80
70
60
50
40
30
20
10
0
84.7
54.9
Extended release
Immediate release
D
Friability (%)
101
100
99
98
97
96
95
Immediate release
Extended release
EP friability limit
0
20
40
60
80
100
Number of rotations
E
Tablet Disintegration Time (min)
250
200
150
100
50
0
243
13
EP Specification of 15 minutes
Extended Release
Immediate release
F
Cumulative drug release (%)
100
80
60
40
20
0
Immediate release
Extended release
0
1
2
3
4
Time (Hours)

Figure 4:

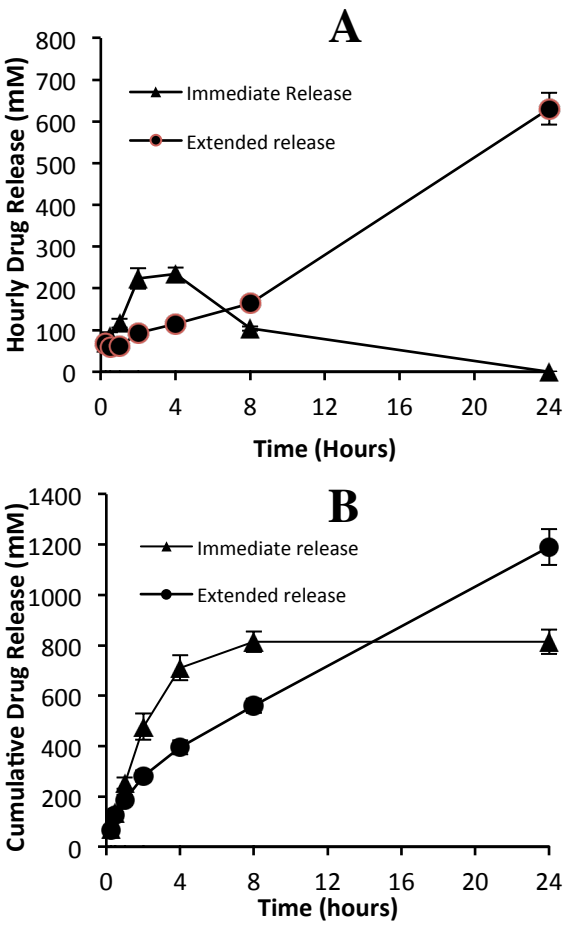

A
Hourly Drug Release (mM)
800
700
600
500
400
300
200
100
0
Immediate Release
Extended release
0
4
8
12
16
20
24
Time (Hours)
B
Cumulative Drug Release (mM)
1400
1200
1000
800
600
400
200
0
Immediate release
Extended release
0
4
8
12
16
20
24
Time (hours)

Figure 5:

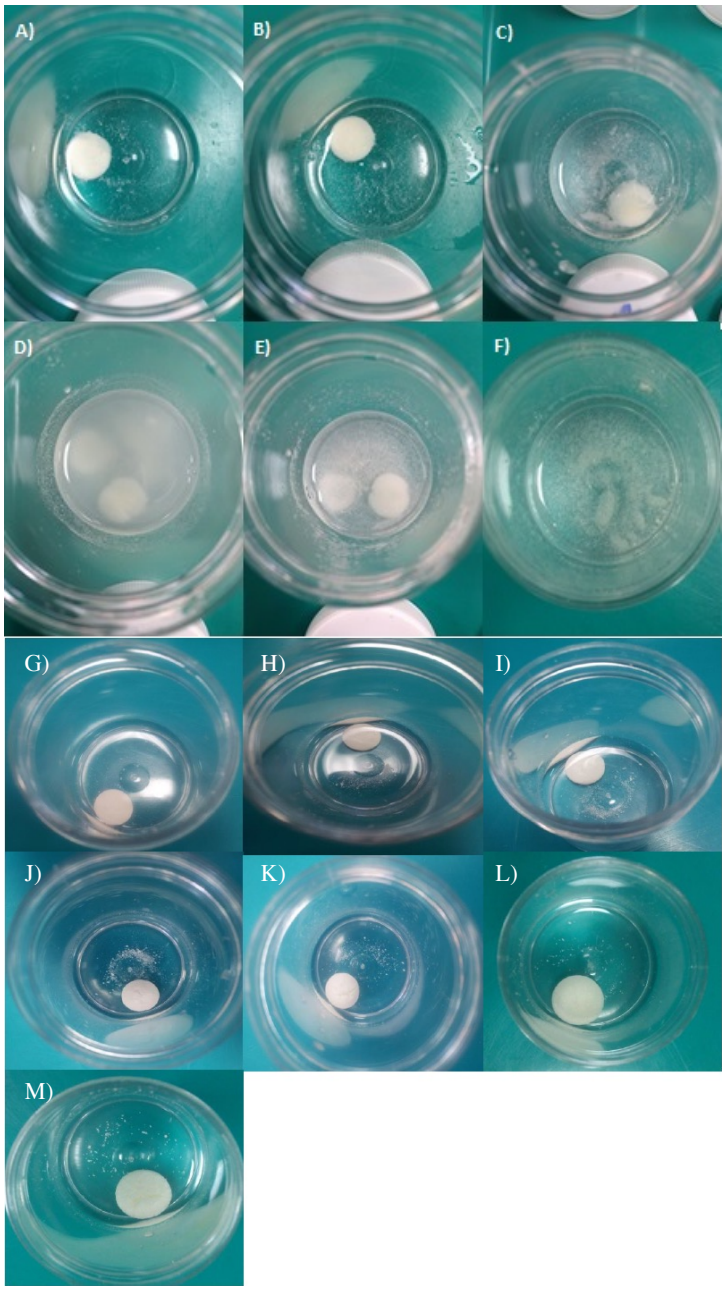


Figure 6:

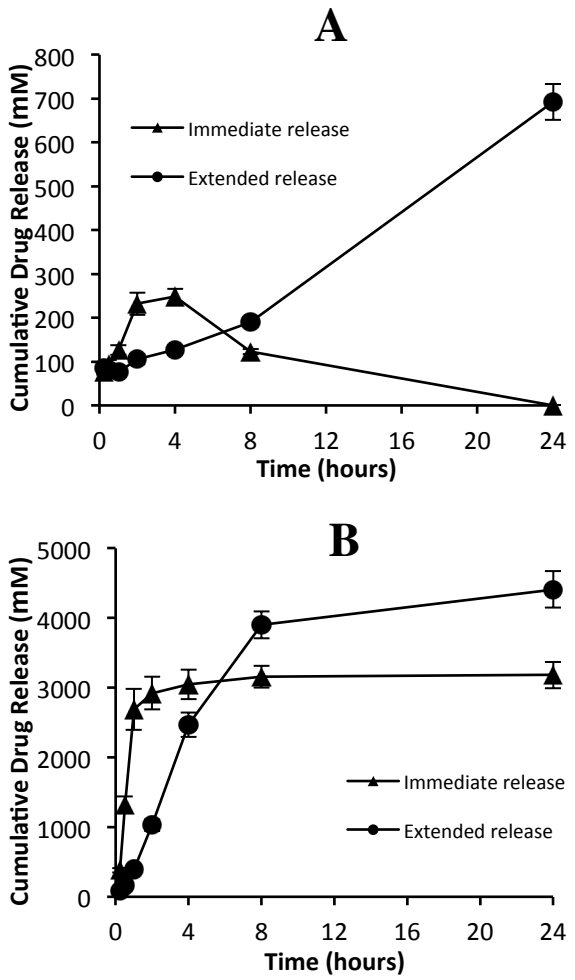

A
Cumulative Drug Release (mM)
800
700
600
500
400
300
200
100
0
Immediate release
Extended release
0
4
8
12
16
20
24
Time (hours)
B
Cumulative Drug Release (mM)
5000
4000
3000
2000
1000
0
Immediate release
Extended release
0
4
8
12
16
20
24
Time (hours)

Table 1:

Ingredient	Immediate release active mix	Extended release active mix
Disulfiram	30.0%	30.0%
Lactose	27.9%	30.0%
Magnesium Stearate	0.6%	2.0%
Kollidon® SR		37.0%
Silicon Dioxide		1.0%
Methyl Cellulose	25.0%	
Stearic Acid	1.5%	
Maize Starch	10.0%	
Microcrystalline Cellulose	5.0%	