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RESEARCH ARTICLE

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Development of Dual Drug Eluting Stent Releasing Paclitaxel and Aspirin in Controlled Manner

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ABSTRACT

Objective

To develop novel formulation for cardiovascular stent with dual drug using biodegradable polymer. Dual drug combination of Paclitaxel and Aspirin will produce anti-proliferative and antiplatelet effects respectively in atherosclerotic artery of human heart by ascertaining controlled release for prolonged action and efficacy.

Experimental/ Computational work done

The Drug Eluting Stent is a novel drug delivery system which delivers the active ingredient or drug to the site of action after implanted to that specific site which is blocked artery due to atherosclerosis leads to angina. The dual drug-eluting stent (DDES) has been developed by incorporating an anti-proliferative and an anti-thrombotic in a biodegradable polymer-coated onto a stainless steel (316 L) stent using spray coating technique. The DDES is prepared by spray coating the bare metal stent with a biodegradable polymer loaded with Paclitaxel and Aspirin, to treat against restenosis and thrombosis, respectively. Biodegradable polymer such as Poly L Lactide and 95/5 Poly DL Lactide co caprolactone were individually formulated with both drugs to get the desired controlled drug release of both drugs. The sterilized DDES were evaluated for various aspects of morphology by gravimetric method, microscopic and SEM evaluation. The drug content and invitro drug release were evaluated by means of HPLC to established required profile to treat against CAD conditions.

Results and discussion

The DDES was formulated using two different combinations with both drugs one using Poly L Lactide and other using 95/5 Poly DL Lactide co caprolactone. The drug polymer interaction was determined by FT-IR and HPLC. HPLC method was developed to perform simultaneously estimation of Aspirin and Paclitaxel. From the morphological evaluation of stent was done using optical microscopy and SEM, it can be conformed that coating texture and surface was very smooth without any cracks, pits or any other irregularities. The drug loading for 11mm and 39mm stents ere also found to be consistent which proved the precision and repeatability of process and analytical method of estimation. The invitro drug release from 11mm and 39mm using Poly L Lactide were also found to be equivalent and desirable to ascertain good quality and safety.

Conclusions

Incorporation of therapeutic agents like platelet aggregation inhibitors like Aspirin and VSMC (Vascular Smooth Muscle Cell) proliferation inhibitors like Paclitaxel into DES may help in reducing the In-Stent Restenosis (ISR) and improving the safety and efficacy of currently available DES. The delivery of multiple drugs would help in the design of promising therapeutic strategies for the treatment of CAD (Coronary Artery disease) using stent based therapies.

ARTICLE HISTORY

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KEYWORDS

Dual Drug Eluting Stent, Paclitaxel, Aspirin

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INTRODUCTION

Over the past decade, drug-eluting stents (DES) have greatly transformed the field of interventional cardiology. Generally, three components are included in a DES system: a metal stent platform, a drug carrier or so called 'stent coating' and a drug. As such, stent coating plays an important role in the performance of DES.[1]

Coronary artery stents, particularly drug-eluting stents (DES), are used in the majority of patients who undergo percutaneous coronary intervention (PCI) to improve symptoms in patients with obstructive coronary artery disease. They function both to prevent abrupt closure of the stented artery soon after the procedure as well as to lower the need for repeat revascularization compared to balloon angioplasty alone (formerly referred to as percutaneous transluminal coronary angioplasty).

Drug-eluting stents (DES) has been well accepted as the treatment for coronary artery blockage. Stents such as the sirolimus and paclitaxel eluting stents have been successful because they reduce the rate of restenosis. However, in spite of the impressive reduction in restenosis, problems remain with the current generation of DES. Attention has shifted to the safety of DES because of evidence suggesting an association with late-stage stent thrombosis. Hence there is a need to develop dual drug eluting stent (DDES), which releases concurrently, both an antiproliferative drug (Paclitaxel) and an anti-platelet drug (Aspirin), incorporated in a biodegradable carrier coated on stent[2].Aspirin has been demonstrated to have antiplatelet properties and is useful in the production of medical devices where it may serve to prevent the binding of thrombocytes (blood platelets) to said medical devices and inhibit the formation of neointima formation (intimal hyperplasia).

The concept of dual-DES therapy is based on the inclusion of a second therapeutic agent, a pro-healing agent to the currently used drugs, which would help in further enhancing the antirestenotic performance of presently available DES. Prof. Kinam Park in his editorial comments gave new insights into the development of dual-DES stating that "the most efficient use of dual DES may come from controlling the drug release kinetics. Ideally, a drug for preventing vascular smooth muscle cells (VSMC) proliferation which is released for the first few weeks, and then the second therapeutic agent promoting re-endothelialization released after a month. Attaining such tailored release kinetics from a thin layer in the range of 5–30 μ on a stent is very difficult. However, with the advent of new technologies in controlled drug delivery we are cautiously optimistic to achieve such tailored and predictable release in the near future". Hence successful performance of dual-DES is majorly based on selection of appropriate therapeutic combination and the regulation of their release kinetics.[3]

This study design represents a dual drug-eluting stent (DDES) that will have an anti-proliferative and an anti-thrombotic in a biodegradable polymer-coated onto a stainless steel (316 L) stent. The DDES will be prepared by spray coating the bare metal stent with a biodegradable polymer loaded with Paclitaxel and Aspirin, to treat against restenosis and thrombosis, respectively. The single or multilayer layered dual-drug coated stent will be characterized in vitro for surface properties.

Paclitaxel is an anticancer drug which helps to prevent restenosis in the artery after stenting. Paclitaxel (PCL), a diterpene amide derived from Pacific yew tree, the most important anticancer agent for the past 18 years. Paclitaxel received FDA approval for the treatment of ovarian cancer in Dec. 1992, metastatic breast cancer in April 1994 and lung cancer. Aspirin is also known as acetylsalicylic acid [ASA], is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication. Aspirin also widely used as an antiplatelet, the effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a patch over damaged walls of blood vessels. Aspirin is part of a group of medications called nonsteroidal anti-inflammatory drugs (NSAIDs), but differs from most other NSAIDs in the mechanism of action.[4,5]

The slow-release formulation of the TAXUS NIRxTM (SR) with drug doase 1μg/mm^2 Stent was safe in this population, and effectively reduced the processes that lead to restenosis. The primary efficacy endpoint was achieved in the TAXUS NIRxTM (SR) Stent treatment group and there were significant improvements versus the uncoated control group for other secondary efficacy endpoints. [6]

In one of the study Aspirin nanofibers on bare metal stents with dose of 1 μg/mm^2, 5 μg/mm^2 and 25 μg/mm^2 were studied to evaluate its effectiveness on platelet adhesion. All three dosed prevent platelet adhesion but as dose increases the effectiveness increase. Hence initially 1μg/mm^2 will be considered for experimentation. [7]

Cardiovascular disease is the dominant cause of mortality in developed countries, with coronary artery disease (CAD) a predominant contributor. The development of stents to treat CAD was a significant innovation. Deployment of a stent overcomes some limitations of balloon angioplasty alone, but provides an acute stimulus for thrombus formation and promotes neointimal hyperplasia. First generation DES effectively reduced in-stent restenosis, but profoundly delay healing and is

susceptible to late stent thrombosis, leading to significant clinical complications in the long term [8].

Drug-eluting stents were developed to lower the rate of restenosis, which now occurs in less than 10 percent of patients treated with these stents. There have been concerns about abrupt thrombosis within drug-eluting stents occurring late after their implantation, leading to acute myocardial infarction and death. Strict adherence to dual antiplatelet therapy with aspirin and athienopyridine is required after stent placement, and the premature discontinuation of therapy is the most important risk factor for acute stent thrombosis. [9]

Platelets are pivotal in the pathogenesis of acute coronary syndrome and in the complications following the implantation of coronary stents. Dual antiplatelet therapy with Aspirin and Clopidogrel is essential to reduce the risk of recurrent ischaemic events. The combination should be taken for up to one year following acute coronary syndrome in patients at high risk of future events. Aspirin should be continued indefinitely. The duration of treatment with Clopidogrel depends on the type of stent implanted. Patients with drug-eluting stents require combination therapy for at least one year. Premature withdrawal of antiplatelet therapy carries a risk of thrombosis in the stent. In patients with drug-eluting stents, thrombosis may occur as a late complication of stent implantation. Withdrawal of antiplatelet therapy should be done in consultation with the cardiologist who implanted the drug-eluting stent. [7]

Patients who've had a stent procedure must take one or more blood-thinning agents. Examples are aspirin and Clopidogrel. These medications help reduce the risk of a blood clot developing in the stent and blocking the artery. Aspirin is used indefinitely. [10]

After implantation of stent the patient has to take Aspirin orally as an antiplatelet action. This research study is design to make drug eluting stent which has Aspirin itself on device to produce the antiplatelet effect from stent surface.

In chemically controlled systems, chemical control can be achieved using bioerodible. The rationale for using biodegradable systems is that the bio erodible devices are eventually absorbed by the body and thus need not be removed surgically. Polymer bioerosion can be defined as the conversion of a material that is insoluble in water into one that is water-soluble. [11]

Hence the produced dual drug eluting stent will serve dual purposes of therapy by generating antiproliferative and antiplatelet activities elicited by Paclitaxel and Aspirin respectively released in controlled manner from biodegradable polymers.

In the present study aim is to develop novel formulation of stent with dual drug effect which will be released in controlled manner. Use of dual drug combination of Paclitaxel and Aspirin will produce anti-proliferative and antiplatelet effects respectively in human heart artery with the use of biodegradable polymers with combination of Paclitaxel and Aspirin to ascertain sustained release for prolonged action and efficacy. Finally, establishment of *in - vitro* drug release kinetics of dual drug.

MATERIALS AND METHODOLOGY

Bare Metalic stent and Paclitaxel were obtained as a gift sample from Sahjanand Medical Technology, Surat. Poly L Lactide and 95/5 Poly DL Lactide co Caprolactone were obtained from PURAC, India. Dichloromethane, Acetonitrile (HPLC grade) and Methanol (HPLC grade) were obtained from Fischer Scientific, India. All other chemicals and solvents used were of analytical grade.

Identification and determination of wavelength (λ_{max})

Scanning of Paclitaxel is to be performed by UV spectrophotometer in Acetonitrile. The 20ppm solution of Paclitaxel was prepared by dissolving 10mg of Paclitaxel in 50mL of Acetonitrile.

Scanning of Aspirin was to be performed by UV spectrophotometer in water. The 20ppm solution of Aspirin is to be prepared by dissolving 10mg of Aspirin in 50mL of water.

UV spectra of both APIs were generated to evaluate the λ_{max}.

Solubility

Both drugs were evaluated in different organic solvents to check their solubility in those respected solvents e.g. Acetonitrile, Methanol, Dichloromethane and water.

Identification of drug by FTIR and HPLC

FT-IR

IR spectroscopy was conducted using a FTIR Spectrophotometer (FTIR-Affinity-1 Shimadzu) and the spectrums were recorded in the wavelength region of400–4000 cm-1. The procedure consisted of dispersing a sample (drug alone and mixture of drug and polymer) in KBr and compressing into discs by applying a pressure of 5 t for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum is to be recorded. All spectras were to be collected at a resolution of 2 cm^{-1}.

HPLC

The specific concentrations of both drugs were prepared in the Acetonitrile. The HPLC method was developed to detect both drugs

simultaneously. The wavelengths at which their UV absorbances are observed maximum were adopted at wavelength of detection in HPLC. The HPLC parameters were optimized for their separation on selected RP 18 column. The drug was identified based in their respective retention time obtained in standard well as in sample.

Preparation of Dual Drug Eluting Stent

The DDES were prepared by spray coating the bare metal stainless steel (316 L) stent with a biodegradable polymer loaded with Paclitaxel and Aspirin, to treat against restenosis and thrombosis, respectively.

Formulations:

The initial trial of batch manufacturing was done using following two formulations

Formulation 01:

Drugs : Paclitaxel and Aspirin (50:50)
Drug Dose : 1 µg/mm^2
Polymer : 95/5 DL Lactide co caprolactone
D:P ratio : 80:20
Total solid contents : 0.3% w/v

Formulation 02:

Drugs : Paclitaxel and Aspirin (50:50)
Drug Dose : 1 µg/mm^2
Polymer : Poly L Lactide
D:P ratio : 80:20
Total solid contents : 0.3% w/v

The methodology for the preparation of proposed dual drug eluting stent was undergone following stages.

Preparation of drug coating solution

The drug coating solution was prepared by dissolving polymers and drug in HPLC grade Dichloromethane. The polymer and drug concentration was taken based drug polymer formulations. The quantity of drug, polymers and solvent were taken as per following table to prepare drug coating solution.

Table 1: Drug coating solution formulation 01 & 02

Formulation 01		Formulation 02	
Ingredients	**Qty**	**Ingredients**	**Qty**
95/5 DL Lactide co caprolactone	0.24 g	Poly L Lactide	0.24 g
Paclitaxel	0.03 g	Paclitaxel	0.03 g
Aspirin	0.03 g	Aspirin	0.03 g
Dichloromethane	100 mL	Dichloromethane	100 mL

Stent coating

Stent coating was done by spray coating technique. All the coating procedure was performed in class 100 clean room having temperature 25±3°C and relative humidity 50±10%.[12]The drug coating solution was sprayed by spray gun on the surface of stent. The specific volume of drug coating solution was sprayed to get the gravimetric weight as following for desired label claim.

Table 2: Stent coating formula 01

Particulars	Value
Stent size	11 mm
Surface area	53 µg/mm^2
Label claim of Paclitaxel	26.5 µg
Label claim of Aspirin	26.5 µg
Gravimetric weight range	260 µg ~ 270 µg

After the specified volume of coating solution required for label claim of drug had been sprayed the organic solvent got evaporated and the layer of drug polymer matrix was formed on the stent surface. Further stent was vacuum dried to remove trace amounts of residual solvents.

Sterilization

The coated stents were sterilized by Gas sterilization technique. The stents prepared were sterilized by ethylene oxide gas by sterilizer. The stents were exposed to EtO gas for specified period of time to get them sterile.

Preliminary trial batch for selection of formulation of DDES

Characterization of DDES system

Weight Uniformity by gravimetric method:

Each stents coated in the batch was evaluated gravimetrically by weighing each stent on microbalance. This is the confirmation test to evaluate the weight uniformity of stents. By this

method it was confirmed the coating is constant to every stent.

Surface characterization of DDES by optical microscopy and SEM:
The surface analysis of coated stent is very important to evaluate any abnormalities in stent coating. The microscopic evaluation was done to check presence of any irregularities like pits, cracks evenness of coating surface. The optical images were taken for reference.Moreover, the SEM images were taken on different magnifications to evaluate the surface morphology precisely.

Identification of drug by HPLC and FT-IR:
As the FT-IR scanning of both drug individually and drug-polymer matrix of both formulations were carried out the FT-IR scanning was not done from stent surface.

Drug content uniformity by HPLC:
The drug content of stents was measured quantitatively by High Performance Liquid Chromatography (HPLC). The coated content was dissolved by taking individual stent in 10mL of volumetric flask. Approximate 9mL of HPLC grade Acetonitrile was added and sonicated for 30min. After sonication the volumetric flask was cooled down to room temperature and volume was made up to the mark with HPLC grade Acetonitrile. Resulting solution was injected in HPLC to estimate the drug content by comparing with known concentration of standard (20 µg/mL).

In vitro drug release kinetic study:
In vitro drug release was analysed in drug release media to be developed and selected based on physiochemical properties of drug and desired *invitro* drug release pattern.Each stent prepared in a lot was analysed for *in vitro* drug release analysis to inspect the controlled drug release pattern. The drug release was analysed in the Phosphate Buffer Saline pH 7.4. The DDES was kept in 10 mL of volumetric flask containing PBS 7.4. The volumetric flask was kept at incubation 37°C at 60 RPM in orbital shaking incubator. After every 24 hrs time interval, the media was replaced with fresh media. The collected media was transferred in 60 mL of separating funnel. Then the solution extracted by 10mL of DCM. The DCM was evaporated and obtained residues were dissolved in 10 mL of HPLC grade Acetonitrile. The resulting solution was analysed by injecting in HPLC and quantified by comparing it with known amount of standard (20µg/mL).

Optimization of batch
In previous study two formulations were prepared i.e. formulation 01 using 95/5 DL Lactide co caprolactone and formulation 02 using Poly L Lactide polymer with dual drugs Paclitaxel and Aspirin.In those formulations, drug lose was observed during spray coating of stent. Hence in next bath the gravimetric weight of stents was optimized by coating 10 % additional amount of matrix to compensate the drug loss during preparation of stent using spray coating method. Hence next iteration of formulation 03 was developed using 11mm and 39mm of stents by following methodology.

Preparation of Dual Drug Eluting Stent
The DDES of 11mm and 39mm were prepared by spray coating the bare metal stainless steel (316 L) stent with a biodegradable polymers loaded with Paclitaxel and Aspirin, to treat against restenosis and thrombosis, respectively.

Formulation
The optimization of batch manufacturing was done by preparing following formulation.

Formulation 03:

Drugs **:** Paclitaxel and Aspirin (50:50)
Drug Dose **:** 1 µg/mm^2
Polymer **:** Poly L Lactide
D:P ratio **:** 80:20
Total solid contents **:** 0.3% w/v

The methodology for the preparation of proposed dual drug eluting stent was undergone following stages.

Preparation of drug coating solution:
The drug coating solution was prepared by dissolving polymers and drug in HPLC grade Dichloromethane. The polymer and drug concentration was taken. The quantity of drug, polymers and solvent were taken as per following table to prepare drug coating solution.

Table 3: Drug coating solution formulation 03

Formulation 03	
Ingredients	**Qty**
Poly L Lactide	0.24 g
Paclitaxel	0.03 g
Aspirin	0.03 g
Dichloromethane	100 mL

Stent coating

Stent coating was done by spray coating technique. All the coating procedure was performed in class 100 clean room having temperature 25±3°C and relative humidity 50±10%.[12]The drug coating solution was sprayed by spray gun on the surface of stent. The specific volume of drug coating solution was sprayed to get the gravimetric weight as following for desired label claim.

Table 4: Stent coating formula 02

Particulars	Value	Value
Stent size	11 mm	39 mm
Surface area	53 µg/mm^2	177 µg/mm^2
Label claim of Paclitaxel	26.5 µg	26.5 µg
Label claim of Aspirin	26.5 µg	26.5 µg
Total Gravimetric weight	295 µg	975 µg
Gravimetric weight range	290 µg ~ 300 µg	970 µg ~ 980 µg

After the specified volume of coating solution required for label claim of drug had been sprayed the organic solvent got evaporated and the layer of drug polymer matrix was formed on the stent surface. Further stent was vacuum dried to remove trace amounts of residual solvents.

Sterilization

The coated stents were sterilized by Gas sterilization technique. The stents prepared were sterilized by ethylene oxide gas by sterilizer. The stents were exposed to EtO gas for specified period of time to get them sterile.

Characterization of optimized batch of DDES system

Weight Uniformity by gravimetric method:

Each stents coated in the batch was evaluated gravimetrically by weighing each stent on microbalance. This is the confirmation test to evaluate the weight uniformity of stents. By this method it was confirmed the coating is constant to every stent.

Surface characterization of DDES by optical microscopy and SEM:

The surface analysis of coated stent is very important to evaluate any abnormalities in stent coating. The microscopic evaluation was done to check presence of any irregularities like pits, cracks evenness of coating surface. The optical images were taken for reference.

Moreover, the SEM images were taken on different magnifications to evaluate the surface morphology precisely.

Identification of drug by HPLC

The identification was active ingredients were done by HPLC by comparing retention times.

Drug content uniformity by HPLC

The drug content of stents was measured quantitatively by High Performance Liquid Chromatography (HPLC).

The coated content was dissolved by taking individual stent in 10mL of volumetric flask. Approximate 9mL of HPLC grade Acetonitrile was added and sonicated for 30min. After sonication the volumetric flask was cooled down to room temperature and volume was made up to the mark with HPLC grade Acetonitrile. Resulting solution was injected in HPLC to estimate the drug content by comparing with known concentration of standard (20 µg/mL).

In vitro drug release kinetic study

In vitro drug release was analysed in drug release media to be developed and selected based on physiochemical properties of drug and desired *invitro* drug release pattern. Each stent prepared in a lot was analysed for in vitro drug release analysis to inspect the controlled drug release pattern. The drug release was analysed in the Phosphate Buffer Saline pH 7.4. The DDES was kept in 10 mL of volumetric flask containing PBS 7.4. The volumetric flask was kept at incubation 37°C at 60 RPM in orbital shaking incubator. After every 24 hrs time interval, the media was replaced with fresh media. The collected media was transferred in 60 mL of separating funnel. Then the solution extracted by 10mL of DCM. The DCM was evaporated and obtained residues were dissolved in 10 mL of HPLC grade Acetonitrile. The resulting solution was analysed by injecting in HPLC and quantified by comparing it with known amount of standard (20µg/mL).

In vitro Drug Release comparison by similarity factor (F2)[13]

In recent years, FDA has placed more emphasis on a dissolution profile comparison in the area of post-approval changes and biowaivers. Under appropriate test conditions, a dissolution profile can characterize the product more precisely than a single point dissolution test. A dissolution profile comparison between pre-change and post-change products for SUPAC related changes, or with

different strengths, helps assure similarity in product performance and signals bioinequivalence. Among several methods investigated for dissolution profile comparison, f2 is the simplest. Moore and Flanner proposed a model independent mathematical approach to compare the dissolution profile using two factors, f1 and f2.

$$f_1 = \{[\Sigma_{t=1}^{n} | R_t - T_t |]/[\Sigma_{t=1}^{n} R_t]\} \cdot 100$$

$$f_2 = 50 \cdot \log \{[1+(1/n)\Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \cdot 100\}$$

Where, Rt and Tt are the cumulative percentage dissolved at each of the selected n time points of the reference and test product respectively. The factor f1 is proportional to the average difference between the two profiles, whereas factor f2 is inversely proportional to the average squared difference between the two profiles, with emphasis on the larger difference among all the time-points. The factor f2 measures the closeness between the two profiles. Because of the nature of measurement, f1 was described as difference factor, and f2 as similarity factor. In dissolution profile comparisons, especially to assure similarity in product performance, regulatory interest is in knowing how similar the two curves are, and to have a measure which is more sensitive to large differences at any particular time point. For this reason, the f2 comparison has been the focus in Agency guidances.

When the two profiles are identical, f2=100. An average difference of 10% at all measured time points results in a f2 value of 50. FDA has set a public standard of f2 value between 50-100 to indicate similarity between two dissolution profiles.

RESULTS AND DISCUSSION

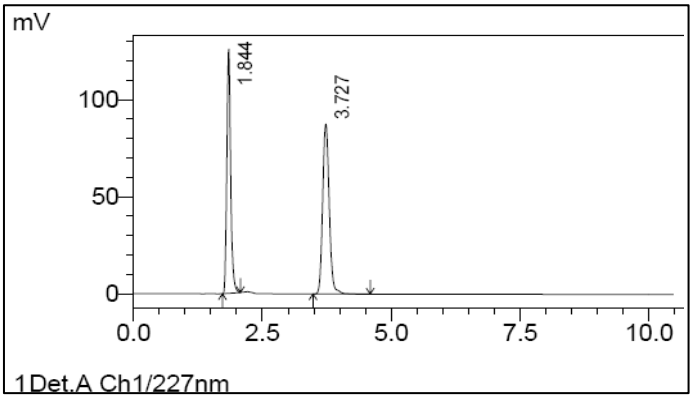
Identification by HPLC

The maximum UV absorbance of Paclitaxel of Aspirin was found to be 227 nm and 226 nm respectively. Hence, 227 nm of wavelength was selected to detect both drugs simultaneously as the UV absorbance at both wavelength does not have significant difference. Table 5 shows HPLC Method Parameters.

Table 5. HPLC parameters

Mobile Phase	Acetonitrile: Water (70:30)
Flow Rate	1.0 mL/min
Column Oven Temperature	40°C
Wavelength of Detection	227 nm
Volume of Injection	20 µl
Run Time	10 min
HPLC column	XTerra RP 18 (250mm × 4.6mm, 5µ)

HPLC Chromatogram



Name	Ret. Time	Area	Height	Area %	Theoretical Plate#	Tailing Factor	Resolution
ASP	1.844	655712	125866	45.780	2430.671	1.268	0.000
PAC	3.727	776601	87564	54.220	3911.527	1.162	9.708
		1432312	213430	100.000			

Fig 1: HPLC chromatogram of Aspirin and Paclitaxel

Identification of drug by Retention time

The stents which were used for drug loading analysis were also evaluated for identification of drug by HPLC. To identify and confirm the presence of drug the retention time of Aspirin and Paclitaxel were compared as obtained in standard and sample

Table 6. RT of drugs in standard

Sr. No.	RT of Aspirin (min)	RT of Paclitaxel (min)
1	1.702	3.747
2	1.699	3.742
3	1.697	3.74
4	1.699	3.743
5	1.69	3.743
Average	1.70	3.74

Table 7. RT of drugs in stent sample

Sr. No.	RT of Aspirin (min)	RT of Paclitaxel (min)
Stent-1	1.647	3.743
Stent-3	1.645	3.741
Stent-7	1.645	3.734
Stent-8	1.64	3.711
Average	1.64	3.73

The retention times of Aspirin and Paclitaxel were found to be 1.7 min and 3.74 min respectively. It can easily be seen that the retention times of both drugs are same in standard and sample.

Characterization of DDES system

Weight Uniformity by gravimetric method:

Table 8. Gravimetric weights of stents after coating of formulation 01

Sr.No.	Initial weight of stent A (µg)	Weight After Coating and vacuum drying B (µg)	Total Loading (A-B) µg	Actual Drug Content (µg)	Actual content of Paclitaxel (µg)	Actual content of Aspirin (µg)
1	13327	13595	268.00	53.6	26.8	26.8
2	13184	13441	257.00	51.4	25.7	25.7
3	13231	13490	259.00	51.8	25.9	25.9
4	13284	13545	261.00	52.2	26.1	26.1
5	13097	13355	258.00	51.6	25.8	25.8
6	13133	13398	265.00	53	26.5	26.5
Avg	13209	13471	261.33	52.27	26.13	26.13
SD	80.60	82.56	3.94	0.79	0.39	0.39
% RSD	0.61	0.61	1.51	1.51	1.51	1.51

Table 9. Gravimetric weights of stents after coating of formulation 02

Sr.No.	Initial weight of stent A (µg)	Weight After Coating and vacuum drying B (µg)	Total Loading (A-B) µg	Actual Drug Content (µg)	Actual content of Paclitaxel (µg)	Actual content of Aspirin (µg)
7	13177	13442	**265**	53	26.5	26.5
8	13219	13484	**265**	53	26.5	26.5
9	13415	13676	**261**	52.2	26.1	26.1
10	13203	13471	**268**	53.6	26.8	26.8
11	13418	13681	**263**	52.6	26.3	26.3
12	13156	13425	**269**	53.8	26.9	26.9
Avg	13265	13530	265	53	27	27
SD	119.58	117.03	2.99	0.60	0.30	0.30
% RSD	0.90	0.86	1.13	1.13	1.13	1.13

Characterization of DDES by optical microscopy and SEM:
Optical Microscopy:

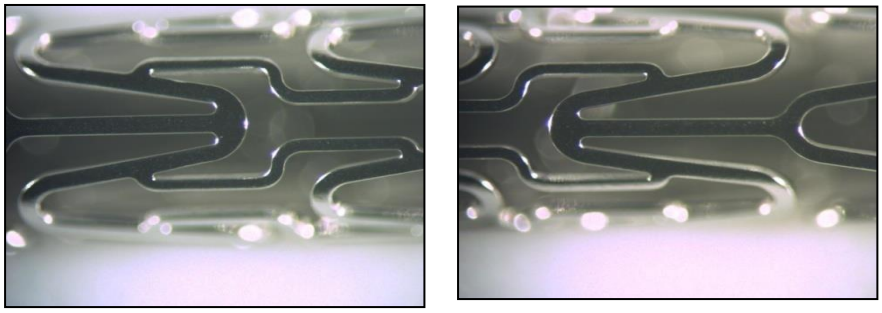


Fig 2. Optical images of coated stents of formulation 01

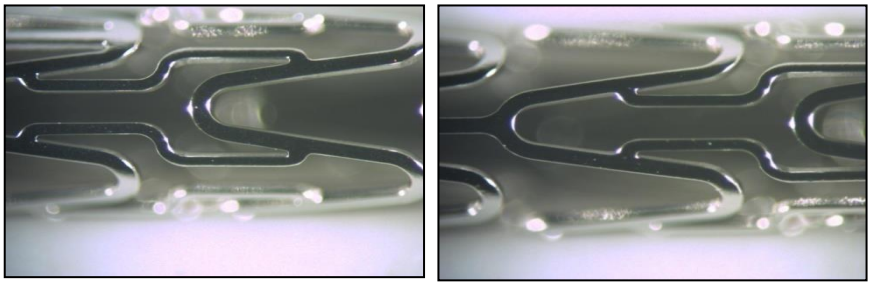


Fig 3. Optical images of coated stents of formulation02

It can be observed that microscopic images show smooth surfaces of stent coating without any irregularities. Hence it can be concluded that the drug coating was done very efficiently by spray coating technique. For further evaluation of coating morphology, SEMs were carried out.

Scanning Electron Microscopy (SEM)

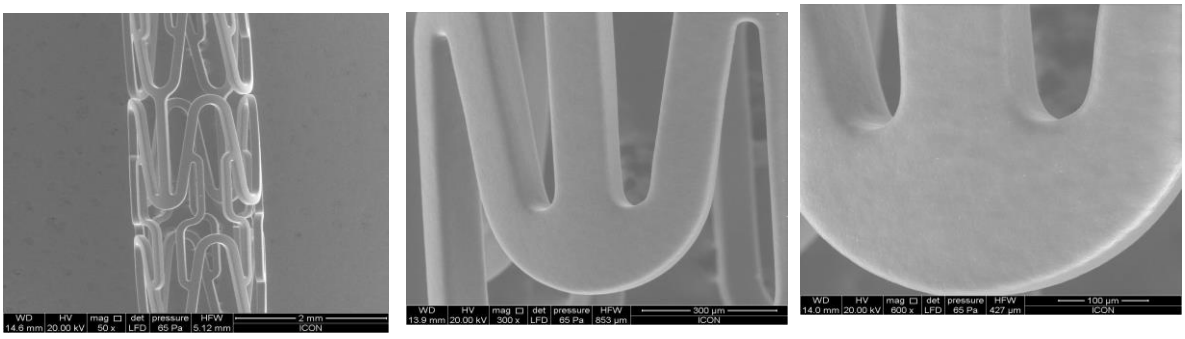


Fig 4. SEM images of coated stents of formulation 01

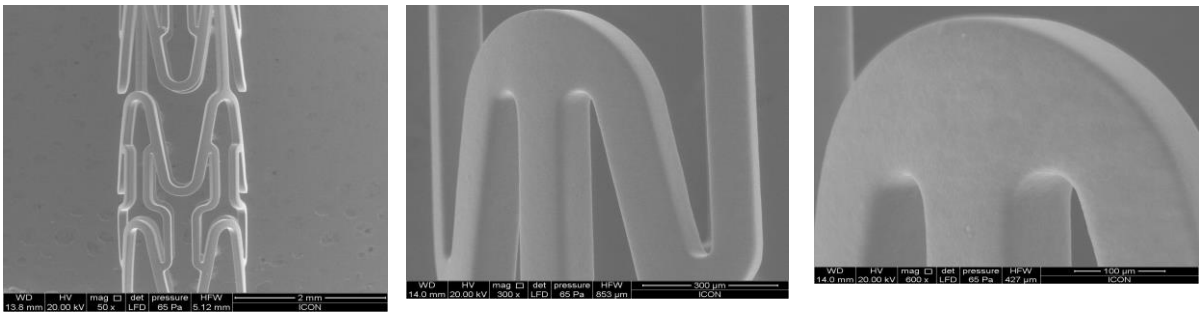


Fig 5. SEM images of coated stents of formulation 02

Scanning Electron microscopy was carried out on stent to evaluate the stent morphology on different magnification i.e. 50X, 300X and 600X. SEM confirms the very smooth texture of coating without any cracks, pits or any other irregularities.

Drug content uniformity by HPLC:

Table 10. Drug content by HPLC of stents after coating of formulation 01

Sr. No	Sample	Gravimetric weight (µg)		Drug Loading (µg)		Factor		% Drug Amount	
		Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
1	Stent-1	26.8	26.8	19.80	20.43	1.35	1.31	74.72	77.09
2	Stent-3	25.9	25.9	19.16	20.64	1.35	1.25	72.30	77.89
	Average			19.48	20.54	1.35	1.28	73.51	77.49
	SD			0.45	0.15	0.00	0.04	1.71	0.56
	% RSD			2.32	0.72	0.09	3.14	2.32	0.72

Table 11. Drug content by HPLC of stents after coating of formulation 02

Sr. No	Sample	Gravimetric weight (µg)		Drug Loading (µg)		Factor		% Drug Amount	
		Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
1	Stent-7	26.5	26.5	20.74	21.24	1.28	1.25	78.26	80.15
2	Stent-8	26.5	26.5	20.38	20.72	1.30	1.28	76.91	78.19
Average				20.56	20.98	1.29	1.26	77.58	79.17
SD				0.25	0.37	0.02	0.02	0.96	1.39
% RSD				1.24	1.75	1.24	1.75	1.24	1.75

The HPLC content of stents of both formulations were carried out by HPLC. The drug content was found consistent by obtaining % RSD 2.32% and0.72% for Aspirin and paclitaxel respectively for formulation 01. For formulation 02, % RSD was obtained 1.24% and 1.75% for Aspirin and paclitaxel respectively. This confirms that the HPLC method shows good repeatability.

In vitro drug release kinetic study:

Table 12. In vitro drug release by HPLC of stents after coating of formulation 01

Day	Sample	Gravimetric weight (µg)		Label Claim (µg)		Drug Release (µg)		%CDR	
		Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
1	Stent-4	26.1	26.1	19.30	20.34	3.47	0.41	17.98	2.02
	Stent-5	25.8	25.8	19.07	20.10	3.67	0.32	19.26	1.61
2	Stent-4	26.1	26.1	19.30	20.34	6.00	0.79	49.08	5.92
	Stent-5	25.8	25.8	19.07	20.10	6.57	0.73	53.70	5.26
3	Stent-4	26.1	26.1	19.30	20.34	1.55	0.97	57.13	10.67
	Stent-5	25.8	25.8	19.07	20.10	1.70	1.02	62.64	10.36
4	Stent-4	26.1	26.1	19.30	20.34	0.66	0.12	60.53	11.28
	Stent-5	25.8	25.8	19.07	20.10	0.64	0.10	66.01	10.83
7	Stent-4	26.1	26.1	19.30	20.34	0.94	0.43	65.42	13.40
	Stent-5	25.8	25.8	19.07	20.10	0.93	0.30	70.91	12.31
8	Stent-4	26.1	26.1	19.30	20.34	0.57	0.17	68.40	14.24
	Stent-5	25.8	25.8	19.07	20.10	0.69	0.14	74.51	13.02
9	Stent-4	26.1	26.1	19.30	20.34	0.72	0.15	72.16	14.99
	Stent-5	25.8	25.8	19.07	20.10	0.79	0.31	78.68	14.57
11	Stent-4	26.1	26.1	19.30	20.34	1.85	0.47	81.73	17.32
	Stent-5	25.8	25.8	19.07	20.10	1.84	0.54	88.30	17.28
15	Stent-4	26.1	26.1	19.30	20.34	0.61	0.18	84.88	18.22
	Stent-5	25.8	25.8	19.07	20.10	0.76	0.21	92.28	18.33
Total	Stent-4					16.38	3.71		
	Stent-5					17.60	3.69		

Table 13. Remaining content of drug by HPLC of stents of formulation 01

Sample	Gravimetric weight (µg)		Label Claim (µg)		Remaining drug content (µg)		Total Drug Recover (µg)		% Drug Recover	
	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
Stent-4	26.1	26.1	19.30	20.34	3.35	15.35	20.95	19.03	108.57	93.59
Stent-5	25.8	25.8	19.07	20.10	3.35	14.70	20.95	18.39	109.82	91.46

Invitro Drug Release Profile

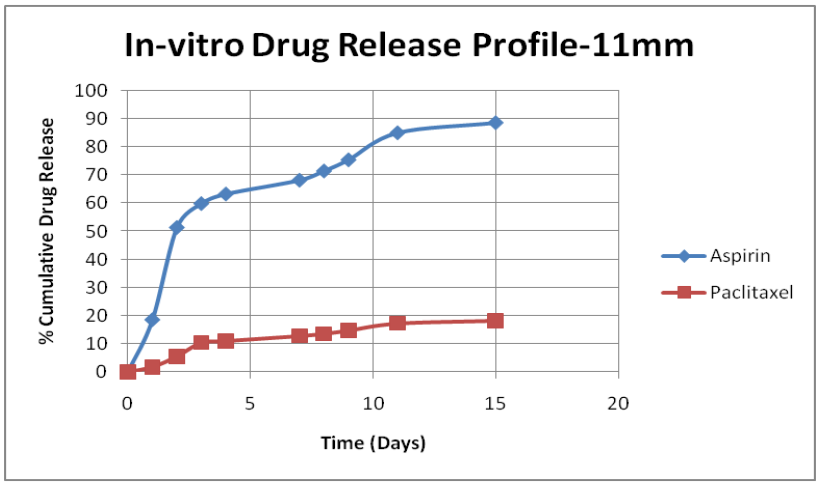


Fig 6. Avg. Drug Release profile- formulation 01

Table 14. In vitro drug release by HPLC of stents after coating of formulation 02

Day	Sample	Gravimetric weight (µg)		Label Claim (µg)		Drug Release (µg)		% CDR	
		Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
1	Stent-9	26.1	26.1	20.25	20.66	4.00	2.00	19.75	9.68
	Stent-10	26.8	26.8	20.79	21.21	4.67	2.10	22.48	9.91
2	Stent-9	26.1	26.1	20.25	20.66	0.98	0.20	24.59	10.65
	Stent-10	26.8	26.8	20.79	21.21	0.85	0.36	26.58	11.63
3	Stent-9	26.1	26.1	20.25	20.66	1.30	1.00	31.03	15.48
	Stent-10	26.8	26.8	20.79	21.21	1.44	0.82	33.52	15.49
4	Stent-9	26.1	26.1	20.25	20.66	1.54	0.27	38.61	16.77
	Stent-10	26.8	26.8	20.79	21.21	1.93	0.27	42.80	16.78
7	Stent-9	26.1	26.1	20.25	20.66	1.37	0.48	45.40	19.11
	Stent-10	26.8	26.8	20.79	21.21	1.44	0.39	49.73	18.63
8	Stent-9	26.1	26.1	20.25	20.66	1.39	0.55	52.25	21.78
	Stent-10	26.8	26.8	20.79	21.21	0.99	0.84	54.51	22.61
9	Stent-9	26.1	26.1	20.25	20.66	0.58	0.10	55.13	22.24
	Stent-10	26.8	26.8	20.79	21.21	0.92	0.06	58.92	22.88
11	Stent-9	26.1	26.1	20.25	20.66	1.66	0.19	63.30	23.15
	Stent-10	26.8	26.8	20.79	21.21	1.53	0.18	66.26	23.71
15	Stent-9	26.1	26.1	20.25	20.66	0.43	0.26	65.45	24.40
	Stent-10	26.8	26.8	20.79	21.21	0.50	0.00	68.69	23.71
Total	Stent-9					13.25	5.04		
	Stent-10					14.28	5.03		

Table 15. Remaining content of drug by HPLC of stents of formulation 02

Sample	Gravimetric weight (µg)		Label Claim (µg)		Remaining drug content (µg)		Total Drug Recover (µg)		% Drug Recover	
	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
Stent-9	26.1	26.1	20.25	20.66	3.26	12.87	17.54	17.90	86.65	86.65
Stent-10	26.8	26.8	20.79	21.21	4.03	13.58	18.31	18.61	88.07	87.72

Invitro drug release profile:

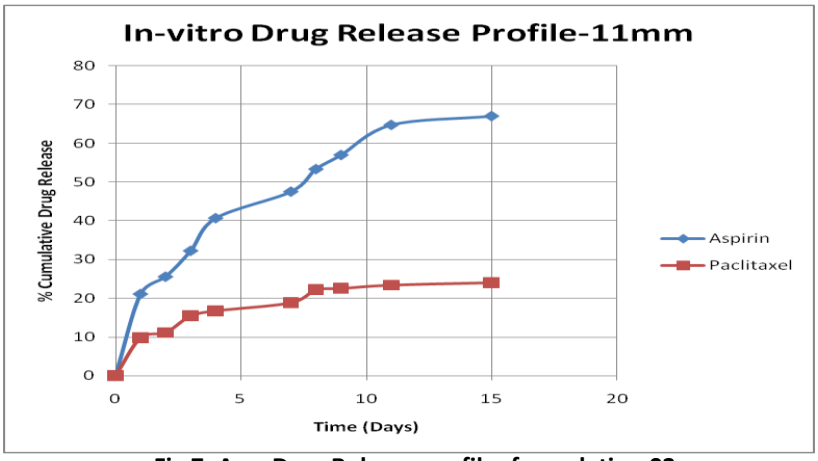


Fig 7. Avg. Drug Release profile- formulation 02

Comparison of Drug Release profiles of both formulations:

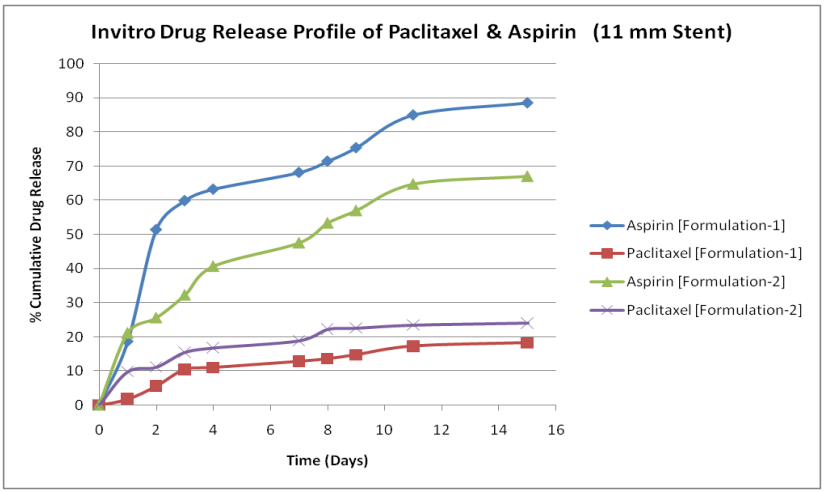


Fig 8. Comparative drug release profiles of formulation 01 & formulation 02

Characterization of optimized DDES batch

Weight Uniformity by gravimetric method:

Table 16. Gravimetric weights of stents after coating of formulation 03 (11mm)

Sr.No.	Initial weight of stent A (µg)	Weight After Coating and vacuum drying B (µg)	Total Loading (A-B) µg	Actual Drug Content (µg)	Actual content of Paclitaxel (µg)	Actual content of Aspirin (µg)
1	13327	13617	290	58	29	29
2	13218	13510	292	58.4	29.2	29.2

3	13229	13520	291	58.2	29.1	29.1
4	13175	13468	293	58.6	29.3	29.3
5	13292	13582	290	58	29	29
6	13418	13709	291	58.2	29.1	29.1
7	13101	13392	291	58.2	29.1	29.1
8	13218	13514	296	59.2	29.6	29.6
Avg.	13247.25	13539	291.75	58.35	29.175	29.175
SD	97.23	96.59	1.98	0.40	0.20	0.20
% RSD	0.73	0.71	0.68	0.68	0.68	0.68

Table 17. Gravimetric weights of stents after coating of formulation 03 (39mm)

Sr.No.	Initial weight of stent A (µg)	Weight After Coating and vacuum drying B (µg)	Total Loading (A-B) µg	Actual Drug Content (µg)	Actual content of Paclitaxel (µg)	Actual content of Aspirin (µg)
1	43022	44000	978	195.6	97.8	97.8
2	42917	43901	984	196.8	98.4	98.4
3	43041	44023	982	196.4	98.2	98.2
4	43104	44080	976	195.2	97.6	97.6
5	43982	44962	980	196	98	98
6	44842	45821	979	195.8	97.9	97.9
7	42164	43143	979	195.8	97.9	97.9
8	38590	39570	980	196	98	98
9	42310	43290	980	196	98	98
Avg.	42593	44541	980	196	98	98
SD	606.70	834.60	2.28	0.46	0.23	0.23
% RSD	1.42	1.87	0.23	0.23	0.23	0.23

Characterization of DDES by optical microscopy and SEM:

Optical Microscopy:

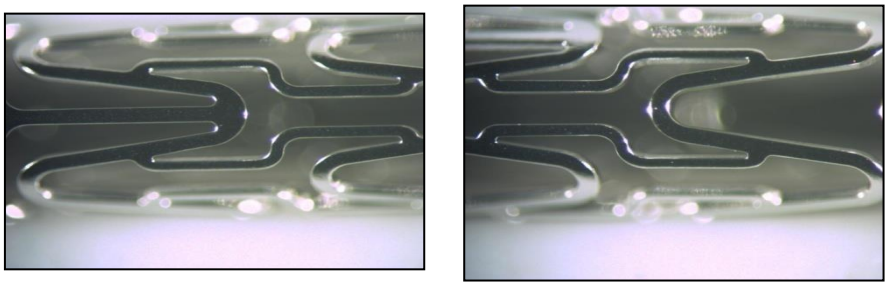


Fig 9. Optical images of coated stents of formulation 03 (11mm)

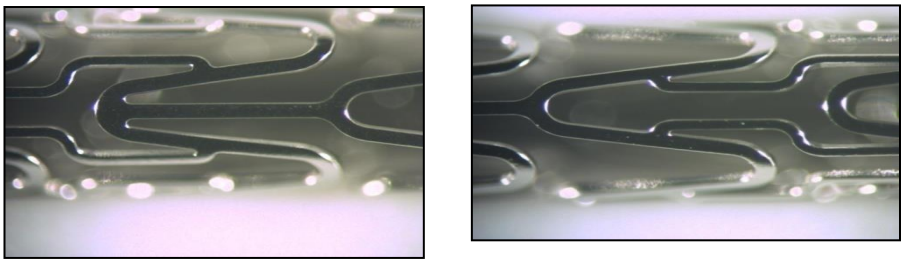


Fig 10.Optical images of coated stents of formulation 03 (39mm)

It can be observed that microscopic images show smooth surfaces of stent coating without any irregularities. Hence it can be concluded that the drug coating was done very efficiently by spray coating technique. For further evaluation of coating morphology, SEMs were carried out.

Scanning Electron Microscopy (SEM):

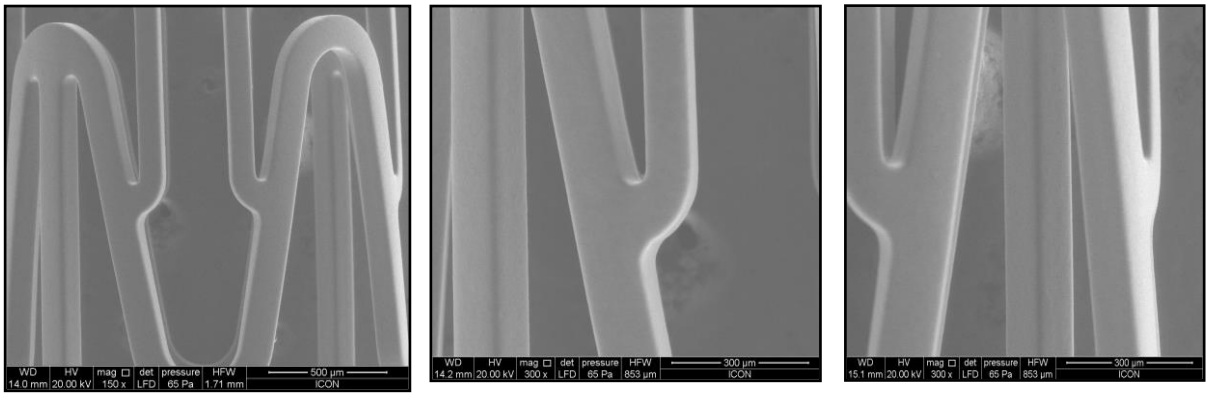


Fig 11. SEM images of coated stents of formulation 03 (11mm)

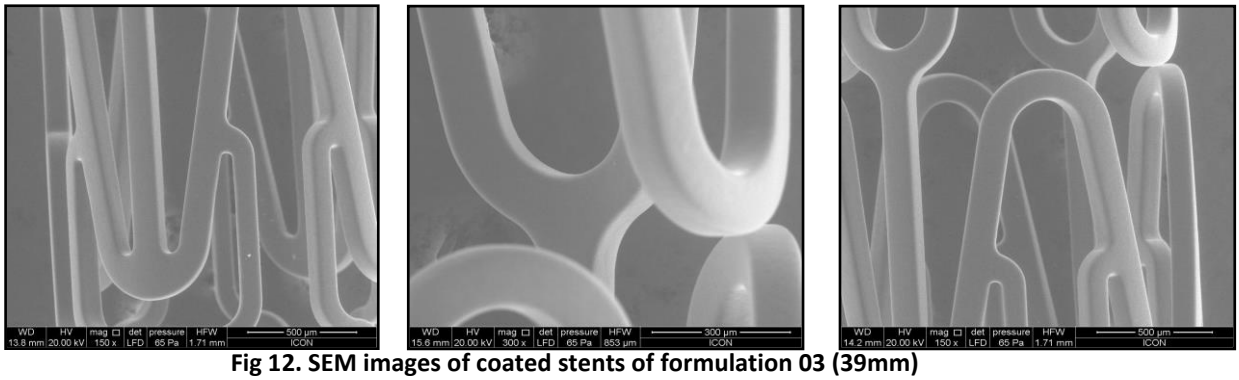


Fig 12. SEM images of coated stents of formulation 03 (39mm)

Scanning Electron microscopy was carried out on stent to evaluate the stent morphology on different magnification i.e. 50X, 300X and 600X. SEM confirms the very smooth texture of coating without any cracks, pits or any other irregularities.

Drug Identification by HPLC

The drug identification from stent samples were carried out by comparison of retention times at which drugs were eluted by HPLC. The comparison of retention time of drug form standard as well as sample of 11mm stents and 39mm of stents. It can be seen that the retention times of Aspirin and Paclitaxel were equivalent or same as obtained in standard and sample. This confirms the identity of drug s in samples.

Drug content uniformity by HPLC:

Table 18. Drug content by HPLC of stents after coating of formulation 03 (11mm)

Sr. No	Sample	Gravimetric weight (µg)		Drug Loading (µg)		Factor		% Drug Amount	
		Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
1	11mm-1	29	29	22.75	22.95	1.27	1.26	84.26	84.99
2	11mm-2	29.2	29.2	21.70	22.30	1.35	1.31	80.36	82.58
3	11mm-3	29.1	29.1	22.67	23.68	1.28	1.23	83.97	87.69
Average				22.37	22.97	1.30	1.27	82.86	85.09
SD				0.59	0.69	0.04	0.04	2.17	2.55
% RSD				2.62	3.00	2.98	3.18	2.62	3.00

Table 19. Drug content by HPLC of stents after coating of formulation 03 (39mm)

Sr. No	Sample	Gravimetric weight (µg)		Drug Loading (µg)		Factor		% Drug Amount	
		Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
1	39mm-01	97.8	97.8	77.32	92.76	1.26	1.05	86.88	104.22
2	39mm-02	98.4	98.4	76.82	89.54	1.28	1.10	86.31	100.61
3	39mm-03	97.6	97.6	77.22	92.21	1.26	1.06	86.76	103.60
Average				77.12	91.50	1.27	1.07	86.65	102.81
SD				0.26	1.72	0.01	0.02	0.30	1.93
% RSD				0.34	1.88	0.75	2.30	0.34	1.88

The HPLC content of stents of both formulations were carried out by HPLC. Considering 10% of process loss the additional 10% matrix content was coated on the stent surface. As a result, the improvement of % recovery of drug was observed. The % drug recovery was found 82.86% for Aspirin and 85.09% for Paclitaxel for 11 mm of stents which was better than the % recovery obtained by formulation 02 i.e. 77.585 and 79.17% for Aspirin and Paclitaxel respectively. The drug contents by HPLC were also found consistent by obtaining % RSD 2.62% (SD 0.59) and 3.0% (0.69) for Aspirin and paclitaxel respectively for 11mm and RSD 0.34% (SD 0.26) and 1.88% (SD 1.72) for Aspirin and paclitaxel respectively for 39mm by formulation 03. This confirms that the HPLC method shows good repeatability.

In vitro drug release kinetic study

Table 19. Invitro drug release by HPLC of stents after coating of formulation 03 (11mm)

Days	Sample	Gravimetric weight (µg)		Label Claim (µg)		Drug Release (µg)		% CDR	
		Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
1	11mm-04	29.3	29.3	22.52	23.12	1.83	0.76	8.12	3.31
	11mm-05	29	29	22.28	22.88	1.80	0.76	8.06	3.34
	11mm-06	29.1	29.1	22.36	22.96	2.00	0.81	8.92	3.53
2	11mm-04	29.3	29.3	22.52	23.12	1.80	0.80	16.11	6.77
	11mm-05	29	29	22.28	22.88	1.75	0.85	15.91	7.05
	11mm-06	29.1	29.1	22.36	22.96	1.90	0.87	17.42	7.32
3	11mm-04	29.3	29.3	22.52	23.12	1.76	0.76	23.93	10.07
	11mm-05	29	29	22.28	22.88	1.82	0.80	24.08	10.55
	11mm-06	29.1	29.1	22.36	22.96	1.85	0.85	25.69	11.02
4	11mm-04	29.3	29.3	22.52	23.12	1.86	0.81	32.19	13.58
	11mm-05	29	29	22.28	22.88	1.70	0.85	31.71	14.26
	11mm-06	29.1	29.1	22.36	22.96	2.00	0.90	34.62	14.94
5	11mm-04	29.3	29.3	22.52	23.12	1.00	0.82	36.63	17.12
	11mm-05	29	29	22.28	22.88	1.75	0.80	39.56	17.76
	11mm-06	29.1	29.1	22.36	22.96	1.60	0.90	41.78	18.86
7	11mm-04	29.3	29.3	22.52	23.12	1.80	0.84	44.63	20.76
	11mm-05	29	29	22.28	22.88	1.77	0.77	47.50	21.12
	11mm-06	29.1	29.1	22.36	22.96	2.05	0.87	50.94	22.65
10	11mm-04	29.3	29.3	22.52	23.12	3.08	0.75	58.29	24.00
	11mm-05	29	29	22.28	22.88	3.12	0.88	61.49	24.97
	11mm-06	29.1	29.1	22.36	22.96	2.98	0.85	64.26	26.35
12	11mm-04	29.3	29.3	22.52	23.12	2.73	0.80	70.42	27.46
	11mm-05	29	29	22.28	22.88	2.72	0.79	73.70	28.42
	11mm-06	29.1	29.1	22.36	22.96	2.76	0.85	76.59	30.06
16	11mm-04	29.3	29.3	22.52	23.12	1.15	0.79	75.52	30.88
	11mm-05	29	29	22.28	22.88	1.09	0.82	78.60	32.01

	11mm-06	29.1	29.1	22.36	22.96	1.08	0.88	81.40	33.89
20	11mm-04	29.3	29.3	22.52	23.12	1.15	0.74	80.61	34.08
	11mm-05	29	29	22.28	22.88	1.09	0.81	83.50	35.55
	11mm-06	29.1	29.1	22.36	22.96	1.08	0.89	86.21	37.77
22	11mm-04	29.3	29.3	22.52	23.12		1.50		40.57
	11mm-05	29	29	22.28	22.88		2.00		44.29
	11mm-06	29.1	29.1	22.36	22.96		1.70		45.17
24	11mm-04	29.3	29.3	22.52	23.12		1.30		46.19
	11mm-05	29	29	22.28	22.88		1.40		50.41
	11mm-06	29.1	29.1	22.36	22.96		1.50		51.70
26	11mm-04	29.3	29.3	22.52	23.12		0.50		48.36
	11mm-05	29	29	22.28	22.88		0.40		52.16
	11mm-06	29.1	29.1	22.36	22.96		0.30		53.01
28	11mm-04	29.3	29.3	22.52	23.12		0.35		49.87
	11mm-05	29	29	22.28	22.88		0.46		54.17
	11mm-06	29.1	29.1	22.36	22.96		0.52		55.28
30	11mm-04	29.3	29.3	22.52	23.12		0.50		52.03
	11mm-05	29	29	22.28	22.88		0.60		56.79
	11mm-06	29.1	29.1	22.36	22.96		0.45		57.24

Table 20. Remaining content of drug by HPLC of stents of formulation 03 (11mm)

Sample	Gravimetric weight (µg)		Label Claim (µg)		RL (µg)		Total Drug Release (µg)		% Drug Recover	
	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
11mm-04	29.30	29.30	22.52	23.12	0.00	7.10	18.15	11.23	80.61	79.29
11mm-05	29.00	29.00	22.28	22.88	0.00	5.60	18.61	12.20	83.50	77.81
11mm-06	29.10	29.10	22.36	22.96	0.00	6.20	19.28	12.29	86.21	80.54

In vitro Drug Release Profile

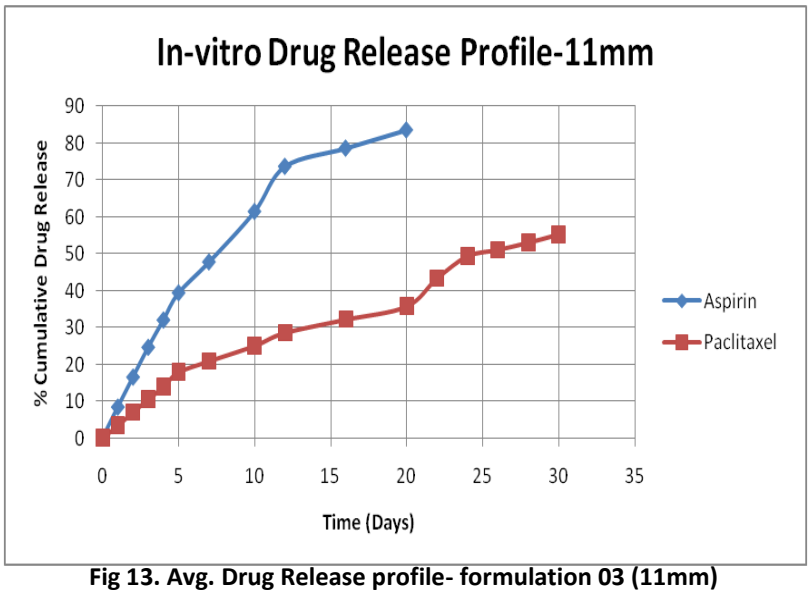


Fig 13. Avg. Drug Release profile- formulation 03 (11mm)

Table 21. In vitro drug release by HPLC of stents after coating of formulation03 (39 mm)

Days	Sample	Gravimetric weight (µg)		Label Claim (µg)		Drug Release (µg)		% CDR	
		Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
1	39mm-04	97.6	97.6	76.86	91.16	7.34	4.16	9.55	4.57
	39mm-05	98	98	77.17	91.54	7.55	4.07	9.79	4.44
	39mm-06	97.9	97.9	77.09	91.44	7.85	4.16	10.18	4.55
2	39mm-04	97.6	97.6	76.86	91.16	7.21	4.15	18.94	9.12
	39mm-05	98	98	77.17	91.54	7.23	4.10	19.15	8.92
	39mm-06	97.9	97.9	77.09	91.44	7.23	4.11	19.55	9.05
3	39mm-04	97.6	97.6	76.86	91.16	6.44	3.68	27.32	13.15
	39mm-05	98	98	77.17	91.54	6.44	3.75	27.50	13.01
	39mm-06	97.9	97.9	77.09	91.44	6.39	3.82	27.84	13.23
4	39mm-04	97.6	97.6	76.86	91.16	6.91	4.03	36.31	17.57
	39mm-05	98	98	77.17	91.54	7.02	4.04	36.60	17.43
	39mm-06	97.9	97.9	77.09	91.44	6.99	4.08	36.91	17.68
6	39mm-04	97.6	97.6	76.86	91.16	9.09	4.68	48.13	22.70
	39mm-05	98	98	77.17	91.54	9.33	4.79	48.69	22.66
	39mm-06	97.9	97.9	77.09	91.44	9.33	4.77	49.01	22.90
9	39mm-04	97.6	97.6	76.86	91.16	7.92	4.22	58.44	27.33
	39mm-05	98	98	77.17	91.54	7.98	4.32	59.03	27.37
	39mm-06	97.9	97.9	77.09	91.44	8.04	4.32	59.43	27.62
10	39mm-04	97.6	97.6	76.86	91.16	7.34	3.61	67.99	31.28
	39mm-05	98	98	77.17	91.54	7.55	3.61	68.82	31.32
	39mm-06	97.9	97.9	77.09	91.44	7.85	3.60	69.61	31.57
12	39mm-04	97.6	97.6	76.86	91.16	7.34	3.61	77.54	35.24
	39mm-05	98	98	77.17	91.54	7.55	3.61	78.61	35.27
	39mm-06	97.9	97.9	77.09	91.44	7.85	3.60	79.78	35.51
16	39mm-04	97.6	97.6	76.86	91.16	4.85	3.18	83.85	38.73
	39mm-05	98	98	77.17	91.54	4.82	3.16	84.86	38.72
	39mm-06	97.9	97.9	77.09	91.44	5.23	2.59	86.57	38.34
20	39mm-04	97.6	97.6	76.86	91.16	0.00	3.20	83.85	42.24
	39mm-05	98	98	77.17	91.54	0.00	3.18	84.86	42.19
	39mm-06	97.9	97.9	77.09	91.44	0.00	3.09	86.57	41.72
22	39mm-04	97.6	97.6	76.86	91.16		3.26		45.81
	39mm-05	98	98	77.17	91.54		3.44		45.95
	39mm-06	97.9	97.9	77.09	91.44		3.24		45.27
24	39mm-04	97.6	97.6	76.86	91.16		3.15		49.27
	39mm-05	98	98	77.17	91.54		3.29		49.54
	39mm-06	97.9	97.9	77.09	91.44		3.18		48.74
26	39mm-04	97.6	97.6	76.86	91.16		3.19		52.77
	39mm-05	98	98	77.17	91.54		3.09		52.92
	39mm-06	97.9	97.9	77.09	91.44		3.38		52.44
28	39mm-04	97.6	97.6	76.86	91.16		3.11		56.18
	39mm-05	98	98	77.17	91.54		3.15		56.36
	39mm-06	97.9	97.9	77.09	91.44		3.18		55.92
30	39mm-04	97.6	97.6	76.86	91.16		3.00		59.47
	39mm-05	98	98	77.17	91.54		3.02		59.66
	39mm-06	97.9	97.9	77.09	91.44		3.12		59.33

Table 22. Remaining content of drug by HPLC of stents of formulation 03 (39mm)

Sample	Gravimetric weight (µg)		Label Claim (µg)		RL (µg)		Total Drug Release (µg)		% Drug Recover	
	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel	Aspirin	Paclitaxel
39mm-04	97.60	97.60	76.86	91.16	0.00	20.25	64.45	50.61	83.85	77.73
39mm-05	98.00	98.00	77.17	91.54	0.00	22.78	65.49	51.00	84.86	80.60
39mm-06	97.90	97.90	77.09	91.44	0.00	21.62	66.74	50.65	86.57	79.03

In vitro drug release profile

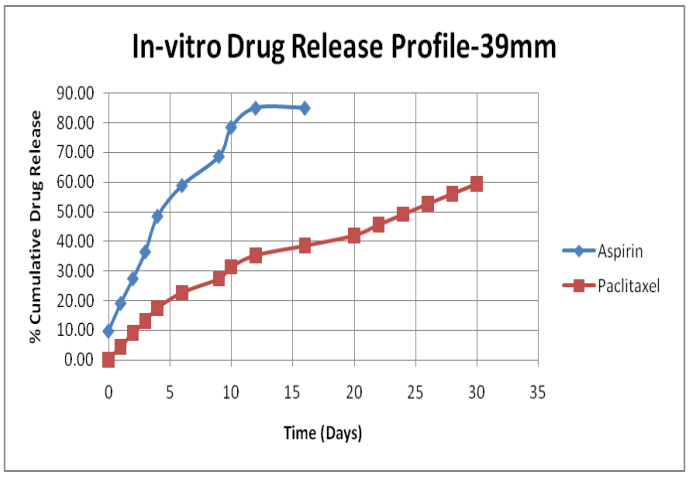


Fig 14. Avg. Drug Release profile- formulation 03 (39mm)

Comparison of Drug Release profiles of both formulations

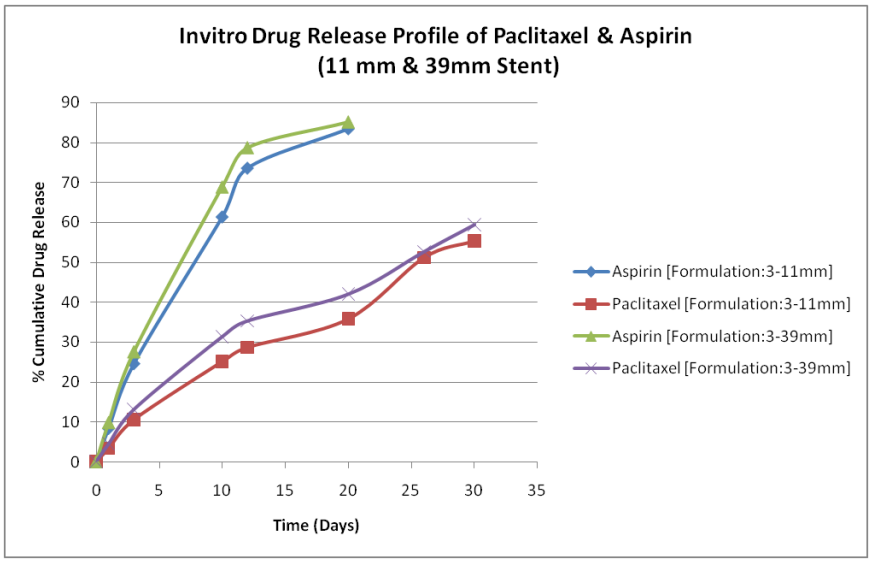


Fig 15. Comparative drug release profiles of 11mm and 39mm stents of formulation 03

Comparison of *In vitro* drug release profile by similarity factor

The equivalency of drug release profiles of drug Aspirin and Paclitaxel obtained from 11mm and 39 of stents were evaluated by calculating the similarity factor between profiles.The individual comparison of Aspirin as well as Paclitaxel was done by calculating F2 values.The f2 value between 50-100 indicates similarity between two dissolution profiles. The similarity factor F2 value was found to be 67 and 68 for Aspirin and Paclitaxel respectively. Henceforth it can be

confirmed that using developed formulation the consistent and equivalent drug release profiles were obtained by two different sizes of stents.

Discussion on drug release profile [13,14]

The coronary stent may induce the appearance of Systemic Inflammatory Response Syndrome (SIRS), where restenosis after stent implantation constitutes the earliest manifestation of an inflammatory reaction. SIRS, the consequent endothelial dysfunction and ischemia are events that follow and are little understood. The inflammatory reaction triggered by the insertion of the stent is caused and maintained by the following factors:

1 - expansion of the stent with rupture of the atherosclerotic plaque and the tunica media
2 - maintenance of the radial pressure of the stent on the arterial wall
3 - the presence of a metallic foreign body
4 - ischemic phenomenon induced by endothelial dysfunction

Mechanical injury and subsequent inflammation of the coronary arterial wall produce the appearance of weak zones, causing the formation of aneurysms, as can be seen in Figure 16.

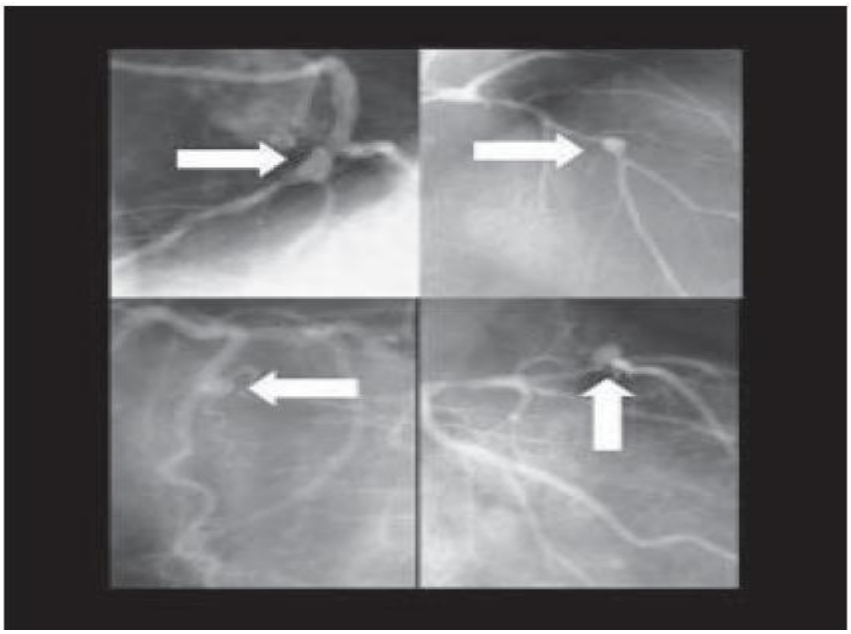


Fig 16. Aneurysms of the coronary arteries after stent implantation

Such inflammatory actions and stent thrombosis event occurs within 10 days after stent implantation if patient is not on antiplatelet therapy after stent angioplasty. Hence antiplatelet or anti-inflammatory response would be required within 10 to 15 days after implantation i.e. of Aspirin and ant proliferative action would be long term initiated by Paclitaxel.Similar kind of drug release pattern has been achieved by the developed dual drug eluting stent in the current study of developing dual drug eluting stents.

CONCLUSION

It was concluded that two formulations were designed to get the controlled drug release of Paclitaxel and Aspirin from the developed DDES. Formulation 01 yields 84.04% of Aspirin and 19.26% of Paclitaxel cumulative drug release in studied *invitro* conditions in 15 days.Formulation 02 yields 67.15% of Aspirin and 25.91% of Paclitaxel cumulative drug release in studied *invitro* conditions in 15 days in case of 11mm of stents. It was also concluded that after stenting body generates inflammatory action to stent considering it as foreign object and due to injury during stenting. Hence anti-inflammatory and antithrombotic action is required within 2 weeks after stenting. Slow and long term release of Paclitaxel is required for antiproliferative action in later stage.Formulation 02 obtained the required drug release pattern, so next iteration was taken to check the reproducibility of formulation 03. Formulation 03 was modified by preparing the stent by coating 10% additional amount of matrix to compensate drug loss during spray coating.After getting the desired profiles of both drugs in 11mm of stents, the formulation was tried on 39 mm of stents to check the consistency and reproducibility. Finally, the equivalency of drug release profiles obtained by 11mm and 39mm of stents, similarity factor F2 was calculated. The similarity factor F2 was obtained to be 65 which proved that the drug release pattern obtained from both 11mm and 39 mm are equivalent to each other. This indicates the

consistency of process as well as release kinetics. Thus, Incorporation of therapeutic agents like platelet aggregationinhibitors like Aspirin and VSMC (Vascular Smooth Muscle Cell)proliferation inhibitors like Paclitaxel into DES may help in reducing the In-Stent Restenosis (ISR) and improving thesafety and efficacy of currently available DES. The delivery of multiple drugs would help in the design of promising therapeutic strategies for the treatment of CAD (Coronary Artery disease) using stent based therapies.

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