NOVEL STRATEGIES FOR OPTIMISING DRUG RELEASE FROM MEDICAL IMPLANTS

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ABSTRACT

In-stent restenosis (ISR) is a significant limitation of percutaneous coronary revascularisation procedures, occurring in around 30% of patients. Implantation of the sent produces an intense inflammatory response. This leads to high levels of smooth muscle cell (SMC) proliferation and migration, resulting in the development of a neointima, which renarrows the artery lumen. Drug-eluting stents (DES) release drugs to inhibit SMC proliferation and have been very successful in reducing ISR rates. However, they are not suitable for every patient group and lesion type, and may induce delayed healing of the endothelium, leading to a risk of late stent thrombosis. There is thus a need to develop more effective drug-eluting stents. Emerging evidence suggests that oxidative stress plays a key role in in-stent restenosis. Polypyrrole is a biocompatible conducting polymer which can act as an antioxidant and is therefore a promising material for use in DES. It can also incorporate anionic molecules during synthesis. Salicylate is an anionic molecule with anti-inflammatory properties and which has also demonstrated anti-proliferative effects on SMC. This project investigated the possibility of creating a polypyrrole stent coating, which has the ability to release salicylate over a therapeutically relevant period of time.

A series of polypyrrole coatings were produced on stainless steel by either potentiostatic or cyclic voltammetry electropolymerisation. The surface characteristics of these coatings was then determined using Scanning Electron Microscopy. Finally, the coatings were immersed in physiological solution and salicylate release quantified at various time points up to 28 days, using UV-spectroscopy.

In this project we found that there may be a relationship between the coating method chosen, the characteristics of the surface and the drug release profile generated. By varying the parameters of the coating process, it should be possible in future to modify the drug release profile of the coating.

CONTENTS

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

A recent WHO (World Health Organization) report, Global atlas on cardiovascular disease prevention and control, states that cardiovascular diseases are the main causes of death and disability in the world (WHO, 2011). In 2008 it has been estimated that 17.3 million people died from cardiovascular diseases representing 30% of all global deaths. According to the WHO work, an estimated 7.3 million of these deaths were due to coronary heart disease and 6.2 million were due to stroke. Over 80% of cardiovascular disease deaths take place in low- and middle-income countries and occur almost equally in men and women.

Atherosclerosis is the leading cause of the cardiovascular diseases (Steinberger & Daniels, 2003). It can be explained as a degenerative disease of the artery, and its fundamental lesion is called atheroma, an aggregation of lipids, connective tissue, fibrous tissue, blood and calcium. After a certain period of time the atheroma can burst, which induces the formation of a thrombus, which can narrow or block the vessel completely. This can also cause the release of microemboli which can in turn block other downstream arteries as is observed in cases of Stroke.

Other than lifestyle choices, such as smoking and a high-cholesterol diet, there are other risk factors like diabetes, hypertension and hypercholesterolemia, which can all contribute to the development of cardiovascular disease. In 2012, the European commission estimated that more than 32 million European Union citizens suffer from diabetes and around 350 million people worldwide, according to the World Health Organisation (WHO, 2011). Those numbers are set to rise by 25 per cent to about 40 million by 2030. Therefore the problem of cardiovascular disease is set to remain a major health problem for years to come (WHO, 2011).

1.2 Coronary artery disease

The coronary circulation supplies blood and oxygen to the heart muscle. Coronary arteries under normal physiological conditions have an auto-regulation mechanism

that maintains a level of blood flow appropriate to the needs of the myocardium. These vessels have a relatively small diameter which means that, if they are affected by atherosclerosis, severe restrictions in flow and even complete vessel blockages can occur. The main consequences of such blockages are angina pectoris or if left untreated, then myocardial infarction. When the cardiac muscle metabolic demand increases, the coronary vessels can multiply their own flow by up to 4 or 5 times through the auto-regulation mechanism (Maggi et al, 1988). Thus there is a blood flow reserve which the heart can use when necessary, such as during periods of intense exercise or extreme stress. However, when the coronary arteries are affected by atherosclerosis and a blockage occurs, this auto-regulatory mechanism cannot compensate, resulting in a lack of oxygenated blood to the myocardium. Without the essential amount of blood, the local myocardial cells break down, the cytoplasm that was in the cells goes to the interstitial fluid and then to the blood stream creating an immune response and the death of the cells (Hansson & Hermansson, 2011). In the worst case, the patient can die, mainly due to complications, such as serious arrhythmias or heart failure that leads to myocardial infarction.

1.3 Coronary Heart Disease Treatments

The precise treatments used for coronary heart disease depend on the symptoms and the severity of the condition. They can be divided into two categories: revascularisation and medical treatment.

1.3.1 Medical treatments

Medical treatments consist of medicines that act to prevent further complications. Some examples can be the use of ACE inhibitors to lower blood pressure, Aspirin to prevent platelets aggregation, Heparin that prevents blood clotting on the surface of plaques in a critically narrowed artery, and Beta-blockers to lower heart rate, blood pressure, and oxygen use by the heart. In the case of severe coronary disease, where the symptoms can no longer be safely managed medically, the patient needs to undergo a coronary artery revascularisation.

1.3.2 Coronary artery revascularisation

There are two ways to re-establish the coronary blood flow through such a revascularisation: through percutaneous coronary intervention (PCI), also called percutaneous trans-luminal coronary angioplasty (PTCA) or through Coronary Artery Bypass Graft (CABG).

1.3.2.1 Coronary Artery Bypass Graft (CABG)

CABG surgery consists of taking a vessel from the same patient and using it to create a new passage for the blood when there is one or more blocked coronary arteries. Bypass is used as a bridge that enables blood to "jump" the obstruction and continue its flow. The main vessels used as bypass grafts are the saphenous vein, the mammary artery and the radial artery (which is used in case of multiple blockages that require bypass). The first successful coronary bypass was performed in 1960 by the surgeon Robert H. Goetz at the Albert Einstein College of Medicine-Bronx Municipal Hospital Center (Goetz et al, 1961). This procedure was the only revascularisation treatment for many years. However, since the invention of PCI, the use of CABG has decreased dramatically, representing less than 30% of all revascularisations. Today, it is only used in certain patient groups that are unsuitable for PCI (including some diabetic and renal patients) and in the case of very high risk lesions or multiple vessel disease.

1.3.2.2. Percutaneus Coronary Intervention (PCI)

The majority of revascularisations are now carried out by PCI, with approximately 90,000 such procedures being carried out annually in the UK alone (BCIS Audit Returns 2010). PCI consists of introducing a balloon catheter into the blocked vessel through the Femoral artery or the Radial artery. The catheter used in this procedure is essentially a tube, small in diameter, with a balloon attached to the end, which when inflated presses the atherosclerotic plaque against the vessel wall and expands the lumen diameter, thus restoring blood flow. At the end of the procedure, the balloon is deflated and removed from the body.

Although many successful revascularisations were performed using this procedure since its introduction in 1977 by Andreas Grüntzig (Grüntzig et al, 1979), it became clear that it was limited by two significant shortcomings. Firstly, in common with many materials when an artery is compressed, it tends to spring back to some degree. The result of this process, known as elastic recoil, is a rapid reduction in luminal area and an increased risk of arterial blockage in a very short period of time. In fact, in one study conducted by Haude et al, the mean elastic recoil after balloon angioplasty was about 31% in diameter and 48% in area (Haude et al, 1993). The second limitation is a vessel remodelling process which takes place in the weeks and months after balloon angioplasty. This contributes to neointima formation and vessel restenosis that can result in a loss of greater than 50% of the original vessel lumen area (Haude et al, 1993).

It was for these reasons that, during angioplasty, a stent was introduced to keep the lumen open over the course of time. Today, the vast majority of PCI procedures are now carried out by stenting.

1.4 Bare Metal Stents

Bare Metal Stent (BMS) are small metallic wire meshes, which are used as scaffolds to maintain coronary vessels open. They are mounted on the balloon catheter in a crimped form to keep the profile as low as possible to let the catheter easily pass through the vasculature to reach the heart. At the stenosis site the balloon is inflated, and it pushes the stent against the vessel's walls. The balloon is then deflated, and removed from the body, leaving the expanded metal stent in place to act as a permanent support to the artery thus reducing immediate elastic recoil (Figure 1.1) from around 31% to 4% in diameter (Haude et al, 1993). In addition, stents have been shown to reduce restenosis rates from around 38% with balloon angioplasty alone, to approximately 17% with stents at 6-month-follow-up (Saito et al, 1999).

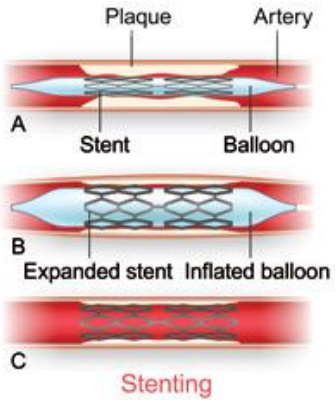


Figure 1.1: Illustration of typical percutaneous coronary intervention (PCI) procedure. The catheter is inserted through the femoral artery and is delivered to the atherosclerotic lesion within the coronary vessel (A). Once in place, the balloon is inflated, causing expansion of the stent and compression of the plaque (B). With the vessel lumen restored, the balloon is deflated and removed, and the stent left in place to provide mechanical support to the vessel wall (C).(Texas Heart Institute. Heart Information centre. Available on http://www.texasheartinstitute.org/ Accessed on 01/08/12).

Treatment with percutaneous BMS implantation provides initial improvements in terms of patient outcomes. There is a reduction in chest pain and an improved quality of life. However, a significant limitation of percutaneous coronary revascularisation procedures is the process of in-stent restenosis (ISR), which is a re-narrowing of the vessel after the implantation (as shown in Figure 1.2).

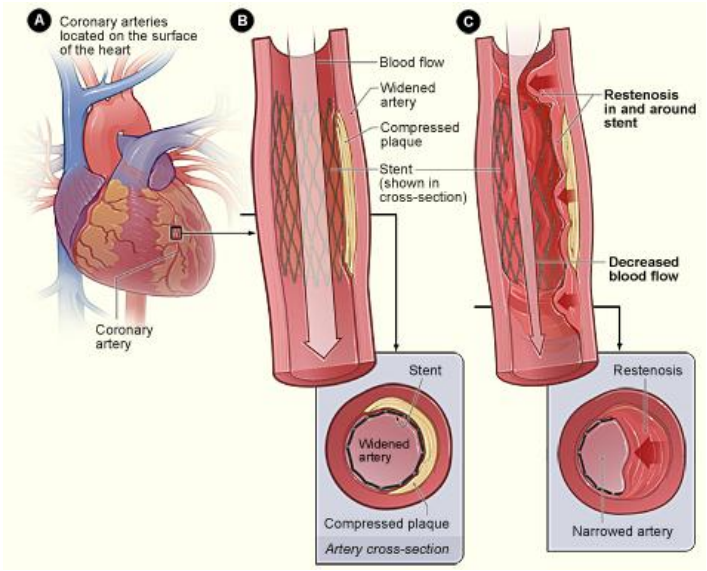


Figure 1.2: (A) Position of the Coronary arteries on the surface of the heart; (B) allocation of a stent into a coronary artery; (C) restenosis in and around the stent (US Deparment of Health and Human Services webpage. Available on http://www.nhlbi.nih.gov Accessed on 01/08/12)

1.5 In-stent Restenosis

In-stent restenosis (ISR) after bare metal stent placement occurs in around 30% of patients (Garg et al, 2010) although the precise figure depends on a variety of patients and lesion specific factors (Ramanath et al, 2010). For example, the presence of underlying disease (in diabetics for example), particular lesion characteristics (highly calcified lesions) and complex lesion geometries (bifurcations) have been shown to limit the effectiveness of stenting as a treatment (Morrison et al, 2007).

In-stent restenosis is a complex process involving a variety of mechanisms. Implantation of the sent into the vessel creates a lesion in the endothelium, leading to early adhesion, aggregation and activation of platelets and thrombus formation. Taken together with the intense inflammatory response that is also produced, this leads to high levels of smooth muscle cell proliferation and migration, resulting in the development of a neointimal layer which re-narrows the artery lumen (Lowe et al, 2002).

It is important to provide some kind of timeline for these events, and most importantly for the overall process of in-stent restenosis. Figure 1.3 showes the timeline of the in-stent restenosis cascade. The first 15 days after stent implantation are characterised by platelet adhesion and aggregation with the final formation of the thrombus. This process causes the recruitment of the leucocytes to the lesion site. Over a period of 90 days after stenting, the inflammatory response leads to smooth muscle cells proliferation and migration and finally to healing and matrix deposition.

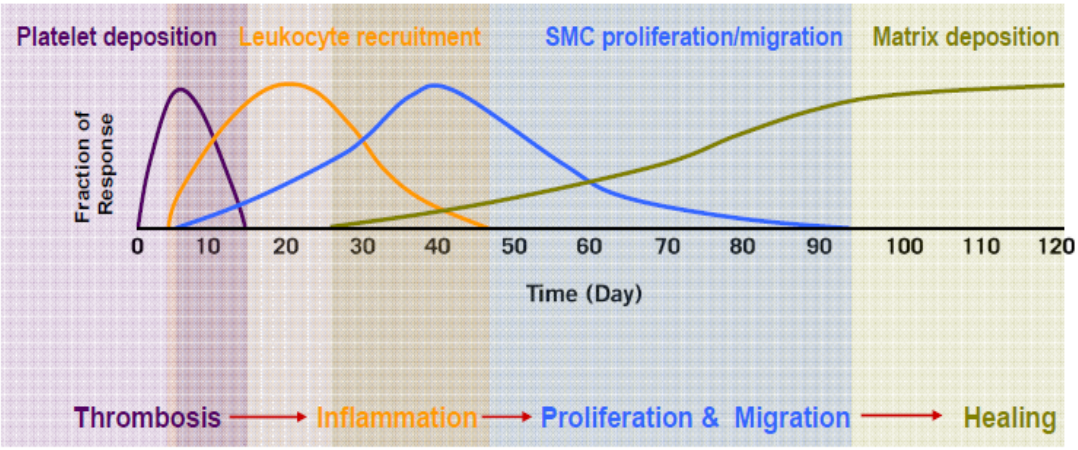


Figure 1.3: Timeline of in stent restenosis cascade. ~15 days after implantation, Platelet adhesion and aggregation/thrombus formation; until ~50 days after implantation, leucocytes recruitment; until ~90 days, smooth muscle cells proliferation and migration; over 120 days, healing and matrix deposition (Source: Forrester et al. J Am Coll Cardiol. 17:758-769, 1991. Welt & Campbell, 2002. Simon, "Inflammation: The Key Element in the Biology of Restenosis." Inflammation Summit. TCT 2003).

In the severest of cases, this re-narrowing leads to a return of the symptoms and a need for a further revascularisation procedure. Several procedures have been used to treat in-stent restenosis. The most common one is a repeated PTCA, but the most recent procedure is Cutting Balloon Angioplasty (CBA), which showed lower rates of restenosis (Serruys & Gershlick, 2005). However, it is clear that any revascularisation is an unwanted procedure for the patient and costly and therefore research efforts were targeted at the prevention of in-stent restenosis.

A wide variety of drugs have been used for the prevention of restenosis, but many showed an inconclusive benefit (Serruys & Gershlick, 2005). The most successful have been the anti-platelet drugs such as aspirin or anti-thrombotic agents such as heparin (Serruys & Gershlick, 2005). Many trials have been conducted to investigate different drug strategies, many of which showed promising results in animal studies, however they did not show the same benefits in human trials. This was commonly put down to the fact that the high drug doses used in animal studies could not be safely used in humans (Serruys & Gershlick, 2005).

Since the level of in-stent restenosis has been shown to be still too high with the bare metal stents, a new kind of stent has been introduced, known as the drug-eluting stent (DES).

1.6 Drug-eluting stents

1.6.1 Introduction

Drug eluting stents (DES) release drugs from the surface of the stent directly into the artery wall, to inhibit smooth muscle cell proliferation, and these have been successful in reducing the level of in-stent restenosis from 30% with BMS to 10% with the first generation of DES (Venkatraman & Boey, 2007). This localized drug delivery system can achieve the high drug concentrations in the surrounding artery wall that are therapeutically effective, whilst avoiding a systemic drug release that could lead to an increased risk of toxicity (Acharya & Park, 2006). In the following section, the current state-of-the-art in DESs will be reviewed. The discussion will initially focus on the first generation of DES developed, the Cypher™ and Taxus® stents, before moving on to second generation devices. The drug delivery mechanisms used in each device will be explored and some of the limitations will be highlighted. Finally, current research within the field will be discussed.

1.6.2 DES. Current state-of-the-art

Several DES have been developed, which differ in their physical, chemical and mechanical characteristics. DES can be broadly classified into two different categories: first and second generation of DES.

1.6.2.1 DES First generation

After several studies which reported the ability of the drug sirolimus (also called rapamycin) to inhibit smooth muscle cell proliferation, a possible role for sirolimus in reducing in stent restenosis was examined (Garg & Serruys, 2010). Despite the fact that early administrations of the drug, orally and locally delivery through special balloons were found to be ineffective, those failures lead to the development of the first DES (Garg & Serruys, 2010). Only in 1999 the first DES implant was performed in humans by J. E. Sousa in Sao Paulo (Sousa et al, 2001). Minimal in stent restenosis was reported at 12 months after implantation (Garg & Serruys,

2010). This research concluded with the development of the Cypher™ stent (Cordis, USA).

Cypher™ is a sirolimus-eluting stent (SES) is a stainless steel stent, coated with polymers which contain the drug, sirolimus. Studies showed that at 1 year follow-up, patients treated with Cypher™ had a rate of binary stenosis equal to 0.0%, compared to the 26.6% of the patients treated with normal BMS (Garg & Serruys, 2010 citing Sousa et al, 2001).

Sirolimus is an immunosuppressive drug that inhibits the cell cycle at the early stage (Venkatraman & Boey, 2007). Poly ethylene-co-vinyl acetate (PEVA) used to coat the Cypher™ stent, is a copolymer made by the combination of ethylene and vinyl acetate and has been widely used in drug-eluting applications (Venkatraman & Boey, 2007). The other components used in Cypher™ are the homopolymer poly butyl methacrylate (PBMA), and a thin coating of parylene, which provides good adhesion between the previous two polymers.

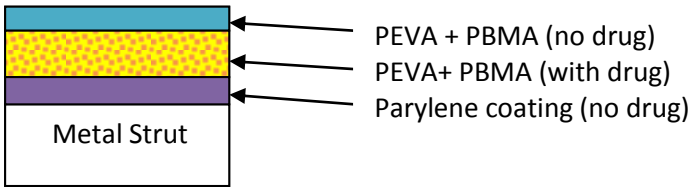


Figure 1.4: Cypher stent configuration with three different layers: Parylene coating in contact with the metal strut; PEVA+PBMA coating with sirolimus; drug-free coat of PEVA+PBMA on the top. Adapted from (Venkatraman & Boey, 2007)

The second DES approved by the FDA was the Taxus® (Boston Scientific) in 2004, after a series of positive clinical trials (Garg & Sarruys, 2010). The Taxus stent has a stainless steel structure and it is coated with a copolymer, a mixer of styrene-b-isobutylene-b-styrene (SIB), which contains the anti-proliferative drug, paclitaxel. Paclitaxel is a microtubule stabilizing agent that inhibits cell cycle at the late stage (Venkatraman & Boey, 2007).

1.6.2.2 DES Second generation

In 2008 Endeavor® (Medtronic Inc.) and Xience™ V (Abbott Laboratories, Inc.) stents were approved by the FDA. Endeavor® uses the Driver bare metal stent (Medtronic, Santa Rosa, CA). This cobalt-alloy stent coated with phosphorylcholine polymer which releases zotarolimus, an analogue of sirolimus (Fajadet et al, 2006). Xience™ V is a cobalt chromium stent coated with a combination of the polymer poly n-butyl methacrylate (PBMA), and PVDF-HFP copolymer, consisting of vinylidene fluoride and hexafluoropropylene monomers which releases an alternative sirolimus analogue, everolimus (Abbott, 2011). This new class of DES introduced an improvement in biocompatibility of the polymer, showing a better degree of re-endothelialisation, compared to the paclitaxel/sirolimus DESs of the first generation (Garg & Serrus, 2010).

1.6.3 DES limitations

Although DES showed better outcomes compared to the BMS (S. Venkatraman & F. Boey, 2007) in terms of in-stent restenosis, several limitations of DES implantation have been reported. It has been suggested that the anti-proliferative drug released by the DES acts on the endothelialisation, inducing delayed healing of endothelial cell layer (Garg et al, 2010). In that case, blood comes in contact with the metallic structure of the stent, leading potentially to increased risk of late stent thrombosis (Garg et al, 2010).

1.6.3.1 Late Stent Thrombosis

Recent studies reported several cases of restenosis and late stent thrombosis in DES. In fact, angiography showed that a year after stent implantation, four patients (two treated with Cypher stents and two with TAXUS stents) had developed in stent thrombosis. Three of the four patients had stopped anti-platelet therapy, so it has been suggested that this lead to the later occlusion of the vessel (McFadden et al, 2004; Venkatraman & Boey, 2007). In 2005 two other cases of in stent thrombosis were reported in patients treated with TAXUS stents at six months after stenting. Even in this case the stenotic events appeared after the interruption of anti-platelet therapy (Venkatraman & Boey, 2007; Nilsen et al, 2006). According to the authors,

this event usually occurs at three months after stenting, but in patients with BMS it has never been reported (Venkatraman & Boey, 2007; Nilsen et al, 2006). From the studies presented above, it is evident how important the duration of the administration of anti-platelet therapy in conjunction with DES implantation is.

1.6.3.2 Hypersensitivity

Another important issue that has been seen to be related to the DES failure is possible hypersensitivity reaction of the patient to specific materials present in the stents. 50 possible hypersensitivity reactions with the Cypher stent have been reported in an article published by Virmani et al in 2004. In particular, a late stent thrombosis case has been observed in a patient who was treated with two overlapping Cypher stents (Virmani et al, 2004). The second stent had been placed 18 months after the previous stenting procedure. The patient presented rashes and irritation three weeks after the second stent implantation. The cause was firstly attributed to the Clopidogrel therapy, but the analysis preformed post-mortem showed the presence of a thrombus in the entrance of one of the two stents. The distal stent presented some fragments of polymer of the stent, surrounded by giant cells. Excluding any hypersensitivity to sirolimus because no cases of hypersensitivity to that drug about have been reported yet, the authors concluded that the hypersensitivity reaction to the polymer presented on the stent was the most likely explanation for the thrombus formation observed (Virmani, 2004; Venkatraman & Boey, 2007). This theory can be confirmed by other cases of hypersensitivity to synthetic polymer. In fact, in a study conducted on rabbits, the polymer n-butyl methacrylate, one of the polymers used to coat the Cypher stents, was seen to provoke hypersensitivity (Revell et al, 1998; Serruys & Gershlick, 2005).

There are only a few studies examining the hypersensitivity response to polymers in humans, whilst there are many such studies on animals in the literature (Venkatraman & Boey, 2007). Animal data obtained from those studies showed that many polymers are suitable for DES, in terms of tissue compatibility (Venkatraman & Boey, 2007).

The concern related to cases of late stent thrombosis with the first generation DES helped inspire the development a second generation of drug-eluting stents. Only the major second generation devices are described here and the reader is referred to a recent review of the field by Garg et al. in 2010.

It has been proposed that one reason for the ineffectiveness of several DES might be mostly related to the inadequate release of the drug. Different mechanisms of drug release and the duration of drug release of DES have been investigated to find the optimal release profile (Raval et al, 2010). Currently Sirolimus, Paclitaxel, Everolimus and Zotarolimus are approved by the FDA, but there are other drugs that are being investigated as potential drugs for use in a DES (Venkatraman & Boey, 2007). To understand if a drug is suitable to be used in DES, the physiochemical properties of the drug and its release profile must be considered (Acharya & Park, 2006). Before developing a new DES, it is important to understand the drug delivery mechanisms currently used in existing DES designs.

1.6.4 Drug release mechanisms

It is very important to have a general knowledge of drug release mechanisms in order to develop new DES. Those mechanisms can be classified into chemical and physical mechanisms. Modifications of the chemical structure of the drug, such us breaking covalent bonds between the molecules, are necessary to bond it to the delivery vehicle presents on the stent. However, drug molecules have had to be modified to obtain the drug-polymer complex, which results in new chemical substances called pro-drugs. Since the action of these new substances may be unknown, it could lead to side effects (Acharya & Park, 2006).

The mechanisms by which drug molecules are released by existing DES consist of diffusion, osmotic pressure, ion exchange and degradation and dissolution of the polymer matrix. The main advantage of these mechanisms is that the delivery system can be designed to control the drug release kinetics. In fact every drug delivery system can be characterised by its specific drug release kinetics and these can be modified by altering specific parameters of the coating, such as type of polymer,

thickness of the coating and the dimension of the exposed surface area (Acharya & Park, 2006).

Diffusion controlled drug delivery systems are the most regularly used, due to their ability to control the drug release kinetics and their easy preparation. Those devices can have two different formulations, and for that reason they can be classified into reservoir devices and the matrix devices.

1.6.4.1 Reservoir devices

In this formulation, the drug is contained in the matrix of the polymer, but a thin polymer layer covers it, acting as a filter which controls the rate of the drug release (Acharya & Park, 2006). Drug release rate is constant at the steady state, so this configuration is characterized by a zero-order release (Acharya & Park, 2006).

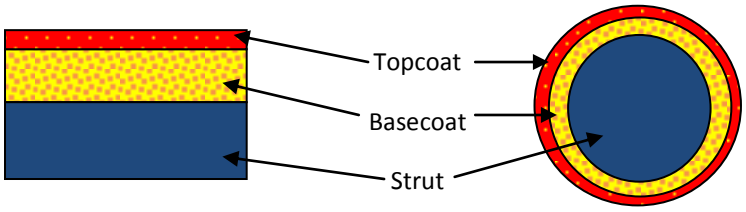


Figure 1.5: Reservoir devices structure with three layers: Strut is covered by a basecoat which contain the drug and the topcoat on top regulates the drug release. Adapted from (Acharya & Park, 2006).

Cypher stent is an example of DESs with this specific formulation. As said previously, Cypher stent has a combination of poly(ethylene-co-vinyl acetate) (PEVA) and poly(n-butyl methacrylate and sirolimus (PBMA) (Acharya & Park, 2006). This coating is covered by a thin PBMA layer that acts as a membrane that modulates the drug release.

Drug release for reservoir devices can be defined by this equation:

$$M=SDK\frac{\Delta C}{h}(t+\frac{h^2}{3D}) \quad (1.1)$$

Where:

- M: Total amount of drug released;
- S: Surface available for drug delivery;
- D: Diffusion coefficient
- K: partition coefficient drug of the membrane;
- ΔC: Concentration gradient;
- H: Thickness of the membrane;
- T: Time of drug release;

With the term $\frac{h^2}{3D}$ representing the initial burst release of drug (Acharya & Park, 2006).

In the in vitro study presented by Venkatraman & Boey, two different Cypher formulations (fast and slow release) were studied. The results showed that the fast release formulation (with no top coat) released the total amount of sirolimus within 15 days, whereas the slow release formulation released the entire amount of drug in 90 days (Venkatraman & Boey, 2007). Both formulations were studied in a human study, which showed different results. In this human study, 30 patients were treated with two different formulations of sirolimus coated stents (slow release and fast release formulations). The slow release formulation was obtained by adding a drug-free layer over the drug-reservoir layer (Sousa et al, 2001; Venkatraman & Boey, 2007). In the 8-months/12-months and 2-years clinical follow-up, no important differences were seen between the two kinds of stents. Although, the slow-release formulation showed an overall better outcome in terms of lumen loss.

The application of the external thin layer is important to reduce the initial burst release which can be toxic, but it also provides enough drug release necessary to avoid neointimal hyperplasia in the first few days after stent implantation.

1.6.4.2 Matrix devices

Stents that have this kind of formulation, are characterized by a layer of polymer and drug, without any other controlling-rate layer. Because drug molecules have no barriers, they leave the polymer matrix directly, resulting in a decreasing rate of drug release over time (Acharya & Park, 2006).

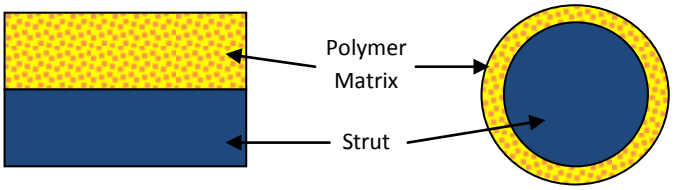


Figure 1.6: Matrix device structure of two layers. The strut is covered by the polymer which contains the drug. Adapted from (Acharya & Park, 2006).

The TAXUS stent is an example of matrix device. The drug release profile of paclitaxel can be explained by this equation:

$$M=S[D\ C_s\ (2A - C_s)\ t]^{\frac{1}{2}} \quad (1.2)$$

Where M, S, D, t are the same terms as previously described for equation 1.

- C_s: solubility of the drug in the polymer;
- A: total drug concentration (Acharya & Park,2006).

1.7 Current Research in Coronary Stents

There is intense research activity, both in academe and industry, targeted at the development of enhanced coronary stents. The most advanced of these approaches are summarised below. However, it is beyond the scope of this review to document each in detail, and the reader is referred to the following comprehensive review articles (Garg et al, 2010; Acharya & Park, 2006). Many approaches have been taken in attempts to improve the biocompatibility of the stent surface. One of the most common approaches was to use biodegradable polymers to coat the stents (Acharya & Park, 2006). Other kinds of active molecules have been investigated for that use such as heparin or fibrin, used to deliver anti-thrombogenic compounds against platelets (Acharya & Park, 2006).

1.8 Oxidative stress and coronary stenting

There is increasing evidence that oxidative stress is produced at the site of stent implantation, in particular the reactive oxygen species (ROS), which may play an

important role in the in development of in-stent restenosis (Azevedo et al, 2000). In a study conducted by Kaminnyi et al, it was observed that the antioxidant Probucol reduced the rate of restenosis after PCI (Kaminnyi et al, 2005).

All mammalian cells have an antioxidant defence system which balances the effect of oxidation. These physiological antioxidants are Glutathione (GSH) and the antioxidant enzyme Glutathione peroxidise (GPx) (Misra et al, 2008).

The role of the Glutathione peroxidise (GPx) is to neutralise hydrogen peroxide (H_2O_2), coupling with it and transforming itself in oxidised glutathione (GSSG) and water (H_2O) (see the reaction below) (Misra et al, 2008).

$$2\ GPx + H_2O_2 \rightarrow GSSG + 2\ H_2O \qquad (1.3)$$

Oxidised Glutathione (GSSG) can regain its anti-oxidative capacity by turning into its previous reduced form. This reaction is catalyzed by the NADPH dependant enzyme, called Glutathione reductase (G-Red).

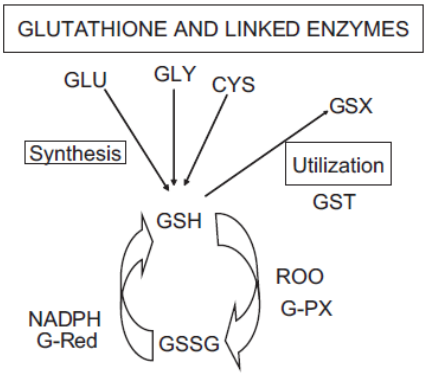


Figure 1.7: Glutathione Oxidation and reduction. GSH indicates glutathione; G-PX, glutathione peroxidase; GLU, glutamic acid; GLY, glycine; CYS, cysteine; GSH, reduced glutathione; GST, glutathione-S-transferase; GSX, glutathione adducts; GSSG, oxidized glutathione; G-Red, glutathione reductase; ROO, reactive oxygen species; NADPH, nicotinamide adenosine dinucleotide phosphate (P. Misra et al, 2008).

There are other endogenous free radicals scavengers, such as superoxide dismutase (SOD) or superoxide catalyse which are able to detoxify reactive oxygen species (ROS), responsible for inducing drastic chemical changes in lipids and proteins

(Misra et al, 2008). That process causes toxicity and consequentially cell damage (Lefer & Granger, 2000).

The role of oxidative stress in in-stent restenosis is still not entirely clear. In the experiments conducted by Misra et al, it had been investigated if the alteration in the levels of anti-oxidative enzymes and oxidative stress agents in the red blood cells (RBC) can be used as a marker in patients with possible in-stent restenosis. The study was conducted on three categories of patients: group A were patients with risk factors of coronary artery diseases; group B were patients with implanted coronary stents but without in-stent restenosis and, finally, group C were patients with implanted coronary stents and evidence of in-stent restenosis. Patients with acute myocardial infarction had been excluded from the study (Misra et al, 2008). It was observed that the Glutatione peroxidise (GPx) level was lower in group C compared to the others. This result suggests that this group was unable to efficiently detoxify lipid peroxidation products, which may have promoted in-stent restenosis in this particular group. The level of Glutathione reductase (G-red) was evident in both groups B and C, suggesting that the presence of CAD contributes to an impaired regeneration of GSH from its reduced form (GSSG). Consequently, high levels of GSSG have been associated with an increased level of oxidative stress (Misra et al, 2008). For that reason, a stent with antioxidant properties may therefore be a significant advance in treatment (Watt et al, 2008).

1.9 Conducting polymers

1.9.1 Introduction

Conducting polymers (CP) were first discovered in the 1960s, but it was not until 1977, after the study conducted on polyacetylene doped with iodine, that their conductive characteristics were recognised (Guimard et al, 2007). In the 1980s another class of conducting polymers emerged, aromatic conducting polymers that showed several interesting properties, such as good stability, conductivity and ease of synthesis (Guimard et al, 2007). The most representative polymers of this type are poly(p-phenylene), polythiophene, polypyrrole, and polyaniline (Vernitskaya & Efimov, 1997).

These kinds of polymers have several characteristics in common with metals, such as electrical and optical properties, but they also present some characteristics of a typical polymer, such as ease of synthesise and processing (Guimard et al, 2007). For that reason CPs can be used in a wide range of applications, such as in photovoltaic and microelectronic industries, electro-chromic display, light emitting diodes and more recently in the biological field (Gurunathan et al, 1999).

The peculiar characteristic of conducting polymers is that they can incorporate specific dopants into the matrix which modify the conductive properties of the polymer. The range of the conductivity for those polymers varies between 10^{-10} to 10^{-5} S cm^{-1}, so when they are doped, their conductivity changes and becomes similar to semiconductors or metals, in the range of 1 to10^{5}S cm^{-1} (Vernitskaya & Efimov, 1997). Doping can be performed through electrochemical oxidation or reduction of the polymer. In case of electrochemical oxidation, the polymer becomes positive, so it can enclose anionic compounds presented in the solution. In the same way, when the polymer is reduced electrochemically, it becomes negative, and so is able to absorb cationic compounds into the matrix (Vernitskaya & Efimov, 1997).

After the discovery in the 1980s that this type of polymer was bio compatible with different biological molecules (Guimard et al, 2007), research in this field increased dramatically. CPs introduced several advantages for biomedical applications over traditional materials such as the ability to entrap and release biological molecules or to transfer charge from a biochemical reaction and the possibility to change easily the intrinsic properties of the CPs according to their specific application (Guimard et al, 2007).

1.9.2 Conducting polymers as coronary stent coatings

In the last few years, conducting polymers have been investigated for a number of possible biomedical applications (Guimard et al, 2007; Arbizzani et al, 2007). Polypyrrole is one of the most studied conducting polymers because of its peculiar physical and chemical characteristics. In fact, it presents very good conductivity and environmental stability, in water and air (Bousalem et al, 2003). It is also easy to synthesise through chemical and electrochemical polymerisation at room

temperature. This polymer was also found to have good biocompatibility, which is an essential parameter for its use in biomedical applications (George et al, 2004; Ramanaviciene et al, 2007). In the study conducted by Gizdavic-Nikolaidis et al., it was demonstrated that this particular type of polymer may act as an antioxidant (Gizdavic-Nikolaidis, 2004). This could have important implications for the use of these polymers as stent coatings, given the potentially negative effect of oxidative stress on outcomes following stenting.

Although conducting polymers have shown to have interesting characteristics, only a limited number of studies (Arbizzani et al, 2007; Okner et al, 2007) have investigated the potential use of the conducting polypyrrole as stent coating. In the study conducted by Arbizzani et al, polypyrrole was used to coat platinum wires through the potentiostatic method of polymerisation. Sodium salicylate was incorporated in the polymer matrix during electropolymerisation and successively its release was measured over a period of time. This study proved the efficacy of this method of coating and the ability of polypyrrole to release sodium salicylate over a certain period of time. However, this study presents several limitations. The platinum wires used in Arbizzani et al experiments did not mimic the common stent strut, which is normally made of stainless steel. The solution used to measure the drug release from the coatings was stored at room temperature, which does not mimic the body temperature (Arbizzani et al, 2007). The method of electropolymerisation used was not compared to other methods available, in order to investigate which one gives the best results. The study conducted by Okner et al. presented the same limitation. Okner et al, had demonstrated the potential of cyclic voltammetry as a polypyrrole coating method (Okner et al, 2007), but a comparison with other coating methods was not made. In the studies seen above no comparisons have been made between the surface characteristics achieved and the drug release profile.

1.10 Project Aims

DESs have introduced several advantages in reducing in stent restenosis rates. However they present several limitations, such as hypersensitivity reaction to the components, delayed healing of the endothelium, leading to a risk of late stent thrombosis. Conducting polymers have been seen to be good candidates for this

application for their particular chemical/mechanical characteristics, but only a few studies (Arbizzani et al, 2007; Okner et al, 2007) have investigated the potential use of the conducting polypyrrole as stent coating, however with some limitations.

Therefore, this project proposes to investigate the possibility of creating a conducting polymer stent coating, which has the ability to release drug over a therapeutically relevant period of time. In particular, the biocompatible conducting polymer polypyrrole will be used to coat stainless steel wires, in order to mimic the stainless steel stents strut. To accomplish this overall objective, the specific aims of this project are:

- To produce a series of conductive polypyrrole coatings using different methods of electropolymerisation.
- To investigate and compare the characteristics of the surface and the drug release profiles of the different coatings.

We hypothesised that the drug release profiles of the wires coated using different methods would be different and that changes in surface characteristics may be related to changes in drug release profile achieved. We also want to investigate the effects of the release medium on the drug release profile.

CHAPTER 2. METHODS

2.1 Introduction

In the first chapter, a number of DES technologies were discussed, focusing on their limitations and the current research work that has been carried out in this field. The chapter concluded by introducing the category of conducting polymers and their possible use as a polymer coating to improve the drug release from stents. A limited number of studies have investigated this particular category of polymers, and assessed their potential use in stent coatings (Arbizzani et al, 2007; Orkner et al, 2007). However, the drug released by the coatings was not ideal and further studies are therefore necessary. In the first section of this chapter, the process of electrochemical polymerisation and methods of measuring drug release performance will both be discussed in detail. This will provide the knowledge necessary to understand the descriptions of the various methodologies adopted in this study, which forms the second and final part of this chapter.

2.2 Electrochemical polymerisation

Conducting polymers can be synthesised through chemical synthesis or electrochemically. Chemical synthesis is performed using a chemical oxidant and it includes different methods of polymerisation, such as condensation or addition polymerisation (Guimard et al, 2007). Electrochemical synthesis is easier to perform and it is a valid alternative to the chemical one (Guimard et al, 2007).

The first electrochemical polymerisation was performed in 1968, with the precipitation of Polypyrrole on a platinum electrode immersed in an aqueous solution of Polypyrrole and sulphuric acid (Guimard et al, 2007 citing Dall'Olio et al, 1968). The precipitation of Polypyrrole was promoted by applying an oxidative potential to the electrodes (Guimard et al, 2007 citing Dall'Olio et al, 1968). Electrochemical polymerisation introduces several advantages, such as the possibility to entrap molecules in the conducting polymer. The procedure is very straight forward and it is possible to achieve thin film synthesis (Guimard et al, 2007). Nowadays, electrochemical polymerisation of conducting polymers is performed using a three

electrodes setup (Figure 2.1) immersed in the solution of the monomer, the solvent and the dopant (Guimard et al, 2007).

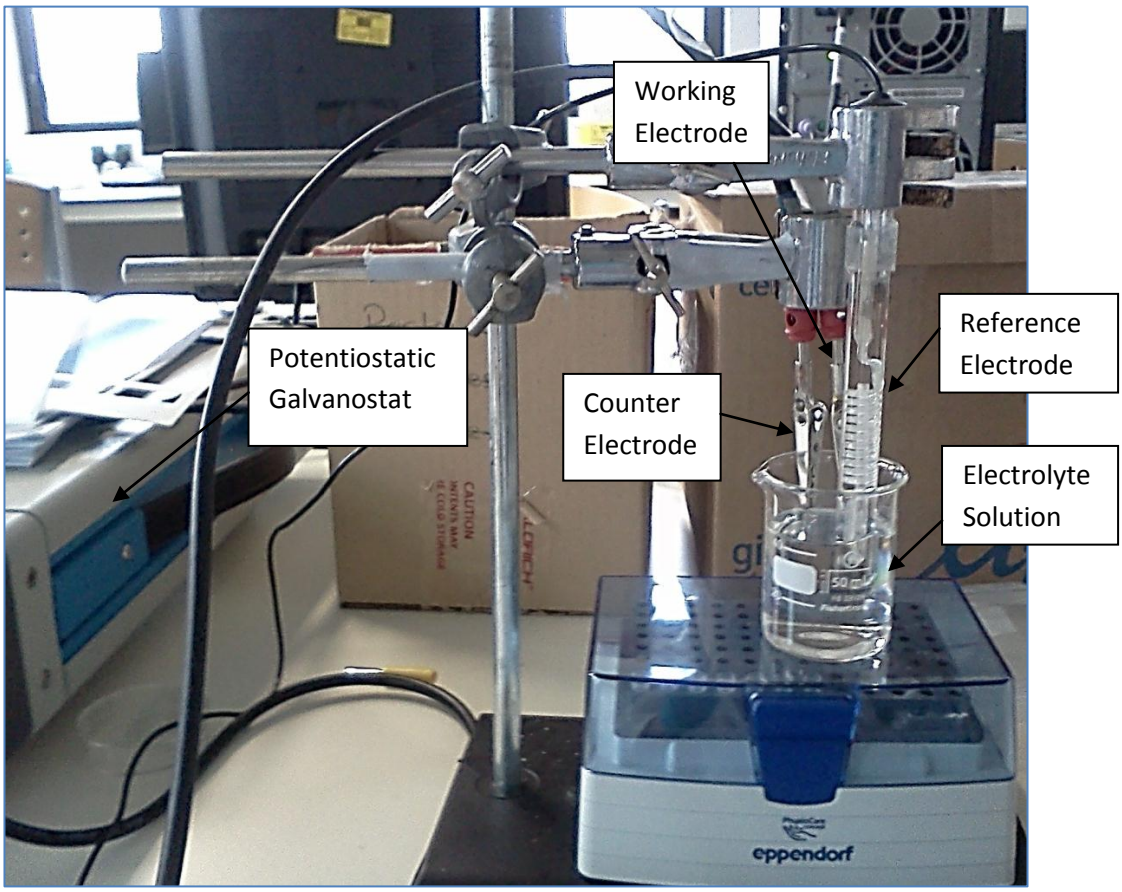


Figure 2.1: Electrodes setup used in the experiments. Current flows from the Galvanostat (showed on the left) to the three electrodes (Counter el., Reference el., Working el.), which are immersed in the electrolyte solution contained in the beaker.

The electrodes consist of a working electrode, where the polymer deposition occurs, the counter electrode and the reference electrode. The electrodes are connected to the potentiostatic galvanostat that provides the voltage necessary to start the process. In fact, current flows from the galvanostat though the solution, charging positively the working electrode and producing the electrodeposition of the polymer on it. Electrodeposition occurs because the monomers present on the working electrode surface become oxidized, forming radical cations that can react with other cations or monomers present in the solution. Those chemical reactions (figure 2.2) lead to the

final creation of an insoluble polymer chain on the surface of the electrode (Guimard et al, 2007).

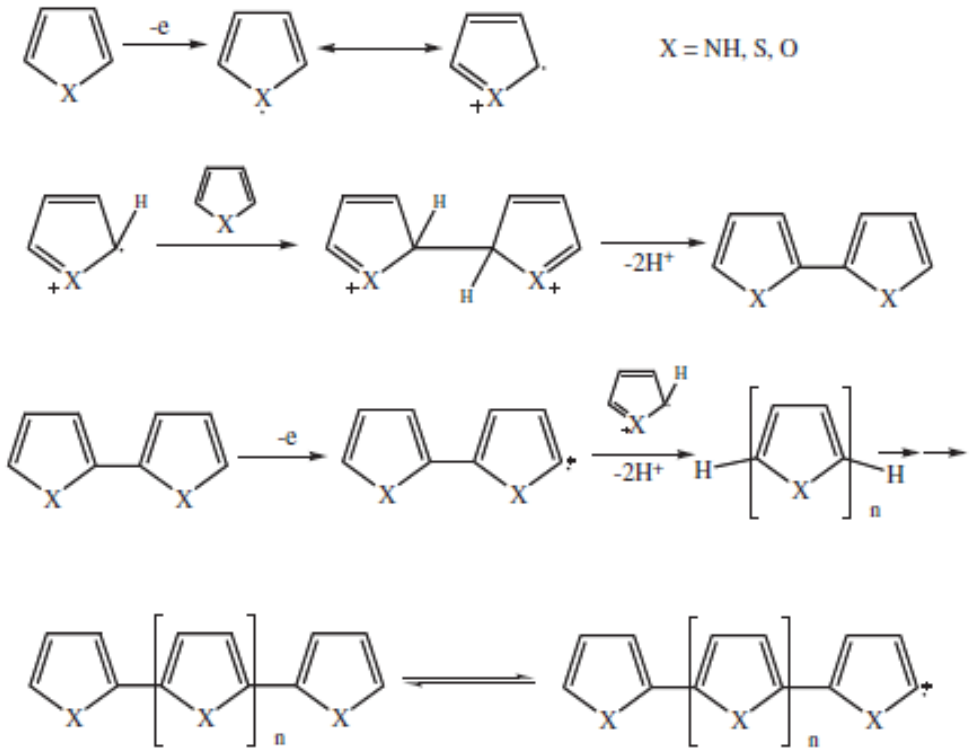


Figure 2.2: Polypyrrole electrochemical polymerisation process. (Guymard et al, 2007).

During oxidation electrons are removed, leaving holes on the polymer. It results in a positively charged polymer, which is then able to incorporate an anionic drug to balance the charge (Svirskis et al, 2010).

Electrochemical polymerisation can be performed using different methods, such as the galvanostatic mode at constant current density, potentiostatic mode at constant potential and the potentiodynamic mode, called also cyclic voltammetry (CV) (Svirskis et al, 2010).

2.3 Drug incorporation during polymer synthesis

During electrochemical polymerisation of the conducting polymer, anions can be incorporated in the polymer structure, to balance the positive charges created by the oxidation. For that reason anionic molecules can combine with the polymer, as

demonstrated by one of the earliest papers in this area, which showed how ferrocyanide, an anionic molecule, could be incorporated in polypyrrole films (Svirskis et al, 2010; Zinger & Miller, 1984). Other drugs have been used as anionic dopants for polypyrrole, including salicylate, naproxene and nicoside anions (Arbizzani et al, 2007; Svirskis et al, 2010; Konturri et al, 1998). These anionic drugs have been then released from polypyrrole films by applying a reducing potential (Svirskis et al, 2010; Konturri et al, 1998).

When a reducing potential is applied post-synthesis, cationic drugs can be incorporated into polypyrrole structure. Several studies (Miller & Zhou, 1987; Hepel & Mahdavi, 1997) showed how cationic drugs, such as dopamine and chlorpromazine can be incorporated into the polymer matrix and then released electrostatically. In those experiments, polypyrrole films were produced in presence of anionic dopants and subsequently reduced to allow cationic drugs to bind to the polymer (Svirskis et al, 2010).

2.4 Parameters affecting electrochemical polymerisation and drug incorporation

Polymer morphology is directly related to the magnitude and the duration of the applied charge during the polymerisation (Svirskis et al, 2010). Constant current used for the electropolymerisation has the advantage to create a homogeneous polymer layer, characterized by good adhesion and mechanical strength (Svirskis et al, 2010). For that reason usually constant current is the preferred method to obtain polymer synthesis. There is a minimum potential that is required to oxidise the monomers, below that value, no synthesis can occur. An example can be found in the study conducted by Boyle et al, 1990, where polymerisation of polypyrrole did not occur with a potential below 0.6V from a solution of 0.5M pyrrole and 0.1M of ATP (Boyle et al, 1990; Svirskis et al, 2010). At low current density or potential, the morphology of the resultant polymer is thinner, smooth and homogeneous (Svirskis et al, 2010). On the contrary, at higher current charge or potential, films are thicker and show a less regular surface with porous structures (Svirskis et al, 2010). In the case of very high potentials, the polymer can undergo overoxidation, resulting in a reduction in adhesion to the electrode and a loss of mechanical and chemical

properties (Svirskis et al, 2010). The size of the dopant molecules have been seen to be also another possible parameter that can change the morphology of the polymer (Shi & Zhitomirsky, 2010).

Another important parameter that must be taken into consideration for the polymerisation is the pH of the electrolyte solution (Svirskis et al, 2010). The pH modulates the speed of the polymerisation, resulting in a fast synthesis in acid conditions, a slower rate in the case of neutral pH and no polymerisation in basic solutions (Svirskis et al, 2010).

Temperature is another parameter that affects the morphology of the resulting polymer (Svirskis et al, 2010). In the study conducted by George et al, polypyrrole was prepared at two different temperatures, at 4° and 25°C, and the results compared. The polymer prepared at the lower temperature showed a more irregular surface, compared to the polymer synthesised at 25°C (George et al, 2005; Svirskis et al, 2010).

As those parameters described above affect the surface characteristics of the polymer, the polymer morphology may influence the drug absorption and release profile achieved. For that reason, by varying those parameters during the electrochemical polymerisation, it may be possible to modify the drug release profile.

2.5 Methods for measuring drug release from DES

Drug release profiles can be measured in different ways, in vivo, in vitro and through computational analysis. With in-vivo testing, DESs are implanted in animals to analyse the behaviour of the DES in an in vivo environment. The pig coronary artery is the recommended model for such evaluations, due to the similar anatomy and physiology between pig and human hearts (Schwartz et al, 2008). However, other animal models can also be used, including the rabbit iliac artery and the rat aorta. In one study conducted by Ma et al. rats were used to investigate the paclitaxel/sirolimus release from a combined DES (Ma et al, 2011). They performed in vivo testing on 30 male rats, placing the DES into their abdominal aorta. At specific time points after stent implantation, rats were sacrificed to harvest the

stented arteries. After having removed the tissue surrounding the stent, the stents were placed in an ethanol/methanol solution and then centrifuged. The concentration of drug was then measured using a UV/VIS spectrometer (Ma et al, 2011).

Whilst drug release profiles measured in vivo are the most accurate predictor of performance in subsequent clinical trials, in vitro testing has been shown to have a valuable role in informing the design of DES. With in-vitro testing, DESs are placed in a solution and samples are then collected over a period of time. The release medium can be a physiological solution, such as PBS, or in cases of sparingly soluble drugs, alternative release media such as ethanol/methanol can be used. Ideally, immersed DESs are stored at 37° C to mimic the in vivo body temperature, although in some cases alternative temperatures are used with many experiments being carried out at room temperature. After having collected the samples for each time point, drug release concentration can be measured by a number of analytical methods, with UV/VIS spectrophotometer being commonly used. Before performing the UV/VIS spectrometry, a standard curve (Absorption versus Concentration) needs to be made by measuring a series of standard concentrations of the specific drug.

The main advantage of this technique is that the measurement can be performed several times, without changing the characteristics of the drug. Concentration of other substances present in the same solution can be measured, varying only the wavelength of light absorption of the particular substance.

Computational studies can be very useful in simulating the behaviour of the DES and the drug release in the artery wall, with the opportunity to vary parameters, such as strut shape, position and coating, under different boundary conditions (Balakrishnan et al, 2005). Therefore, while animal and clinical studies are essential to ensure device safety, mathematical modelling can certainly help to inform the development of DES and aid in our understanding of their potential limitations.

2.6 Polypyrrole coating production

2.6.1 Equipment

Solarton SI 1287 Electrochemical Interface Potentiostat/Galvanostat (Solarton Analytical, Hampshire, UK) was used to perform electrochemical polymerisation of pyrrole (figure 2.1). A three electrode configuration was used, with a Stainless Steel wire as Working Electrode (WE), a Platinum wire as Counter Electrode (CE) and a KR5 Reference Electrode (figure 2.1). 1mm diameter SS316L metal wire was used to simulate the stent in the experimental work. The KR5 reference electrode was purchased from ThermoScientific UK Ltd, Leicestershire, England (Platinum and stainless steel wires were purchased from Goodfellow Cambridge Ltd (Huntingdon, UK). The Electrochemical Interface instrument was controlled by a desktop computer, using CorrWare software. CorrView software was used to plot the resultant graphs.

2.6.2 Materials

Pyrrole (C4H5N, 98% purity, reagent grade, Mw = 67.09g/mol), Sodium salicylate (HOC6H4COONa, ≥99.5% purity, ReagentPlus®, Mw=, 160.10g/mol), Sodium Chloride (NaCl, Mw = 58.44g/mol), Ethanol (CH3CH2OH, reagent grade, Mw = 46.07 g/mol,), were purchased from Sigma-Aldrich. Distilled water was used to prepare the solutions.

2.6.3 Methods

A 500ml solution of 0.1M NaCl 0.1M Py and a second 500ml solution of 0.1M NaSa 0.1M Py were prepared for the electropolymerisation, using an existing Pyrrole stock solution. NaCl is a well-studied and characterised dopant ion (Petit et al, 1999) and for that reason it was used in the preliminary work, in order to establish that the coating could be produced successfully using it. A second set of the same solutions were subsequently prepared following the purchase of a new batch of Pyrrole from Sigma-Aldrich. Solarton SI 1287 Electrochemical Interface Potentiostat/Galvanostat was used for the electropolymerisation.

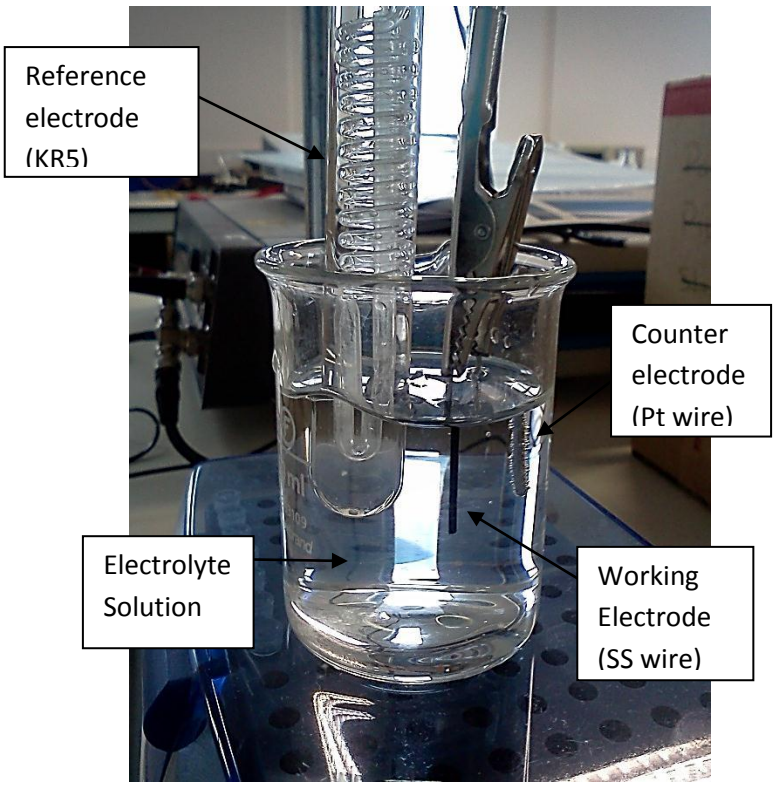


Figure 2.3: Three electrodes setup during electropolymerisation.

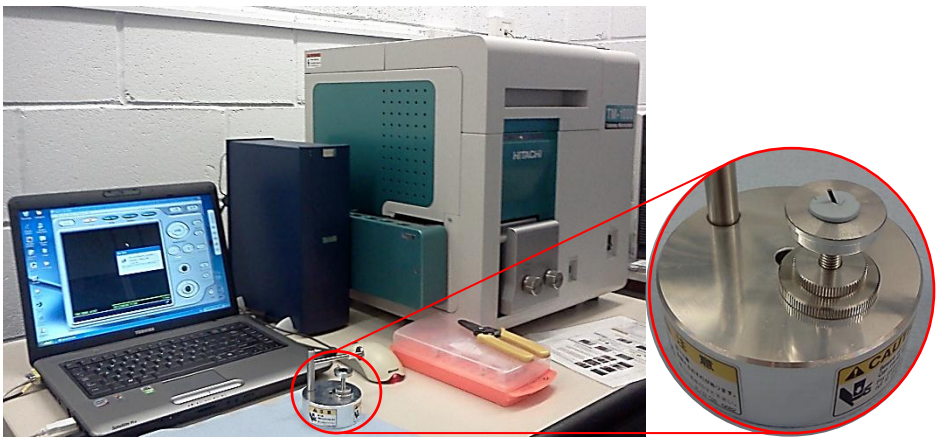
Before performing the electropolymerisation for each experiment, the working electrode and the counter electrode were washed in ethanol and left to dry. The reference electrode was cleaned with distilled water before and between experiments. The electrodes were then immersed in a 40ml solution of 0.1M Pyrrole containing either 0.1M NaSa or 0.1M NaCl as shown in figure 2.3.

Two different types of electrochemical techniques were used in this experiment to coat the wires: Potentiostatic and Cyclic Voltammetry. The Potentiostatic electropolymerisation was carried out at a potential of 0.9V versus the reference electrode for 5 minutes. Cyclic Voltammetry electropolymerisation was set up to cycle between potentials of 0.5V and 2V. Several wires were coated using both methods, and 5 wires for each experiment were then used in subsequent imaging analysis and drug release measurement studies.

2.7 SEM analysis

2.7.1 Equipment

A Scanning Electron Microscope (SEM) (HITACHI TM-1000 Tabletop Microscope) was used to investigate the surface properties of the coatings.



(A) (B)

Figure 2.4: (A) Scanning Electron Microscope (SEM) (HITACHI TM-1000 Tabletop Microscope) used for the experiments. (B) Zoomed image of the wire (cut wire) placed on the disc for SEM imaging. The height of the wire was measured before performing the SEM analyses.

2.7.2 Methods

Polypyrrole coated stainless steel wires coated previously by electropolymerisation were imaged at a variety of different magnifications, ranging from 30x to 6000x. In addition, a number of wires were cut into two pieces (section 2.6), in order to obtain images of the cross sectional area of the coating. A list of wires used in this experiment and their respective details are shown in the table 2.1.

Number of wires	Coating method	Coating Solution	SEM images
1	Potentiostatic	0.1M NaCl 0.1M Py (existing Py)	Surface Cross- section area
1	Potentiostatic	0.1M NaSa 0.1M Py (existing Py)	Surface Cross- section area
1	Cyclic Voltammetry (CV)	0.1M NaSa 0.1M Py (existing Py)	Surface Cross- section area

1	Potentiostatic	0.1M NaSa 0.1M Py (Fresh Py)	Surface Cross- section area
1	Cyclic Voltammetry (CV)	0.1M NaSa 0.1M Py (Fresh Py)	Surface Cross- section area

Table 2.1: Wires used in the experiment and their respectively details (Coating method, Coating solution, SEM images).

2.8 Drug release measurement

2.8.1 Equipment

A UV 2401 PC UV/VIS Recording Spectrophotometer was used to investigate the concentration in the samples. UV-Cuvette UV-Transparent Spectrophotometry Cuvettes were purchased from BrandTech Scientific, inc (Essex,CT). A standard oven was used to store the wires in the solution at 37°C.

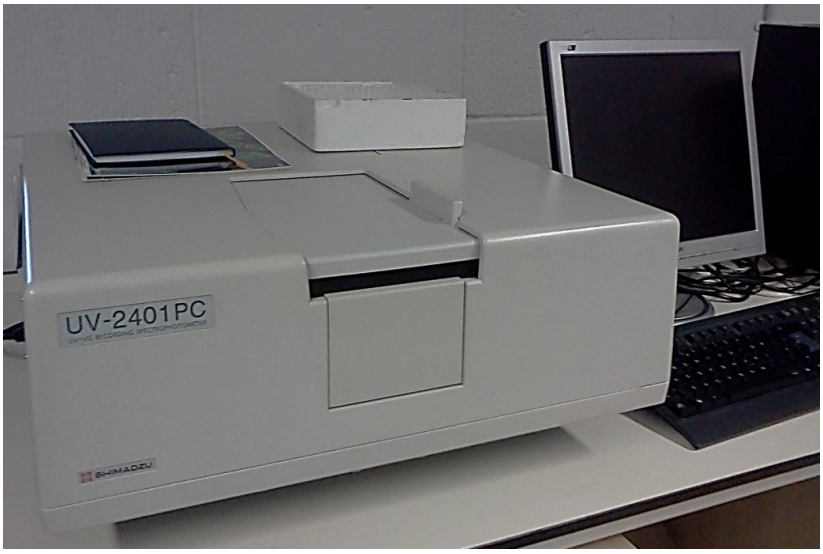


Figure 2.5: UV 2401 PC UV/VIS Recording Spectrophotometer used in the experiments.

2.8.2 Material

Phosphate Buffer Solution was purchased from Sygma-Aldrich. Methanol (CH3OH, reagent grade, Mw= 32.04g/mol, Min Assay (GLC) 99.5%) was purchased from Bamford Laboratories. Distilled water was used to prepare the solutions.

2.8.3 Methods

2.8.3.1 Calibration curve

A stock of 100ml of NaSa at $1x10^{-2}$ M concentration was made by diluting 160.1mg of NaSa into 100ml of 0.1M PBS. This highly concentrated solution was used to form standard solutions necessary for the generation of the calibration curve. This process was repeated until a calibration curve for salicylate was created for a concentration range of 10µM to $1x10^{-3}$M. The table below shows the procedure used to create the samples.

Standard Concentration	**Standard Creation Procedure**
0 M (PBS)	0.1M PBS in dH_2O
$1x10^{-5}$ M	5µl of $1x10^{-2}$ + 4995µl of PBS
$3x10^{-5}$ M	15µl of $1x10^{-2}$ + 4985µl of PBS
$1x10^{-4}$ M	50µl of $1x10^{-2}$ + 4950µl of PBS
$2x10^{-4}$ M	100µl of $1x10^{-2}$ + 4900 µl of PBS
$4x10^{-4}$ M	200µl of $1x10^{-2}$ + 4800 µl of PBS
$6x10^{-4}$ M	300µl of $1x10^{-2}$ + 4700 µl of PBS
$8x10^{-4}$ M	400µl of $1x10^{-2}$ + 4600 µl of PBS
$1x10^{-3}$ M	500µl of $1x10^{-2}$ + 4500 µl of PBS
$1x10^{-2}$ M	160.1mg of NaSa + 100ml of PBS

Table 2.2: Calibration curve PBS Standards

A new calibration curve was produced for each day on which sample analyses were performed. Standards used to produce calibration curves were stored at room temperature. The same methodology was used to produce the standard solutions for the drug release measurement in methanol. In this case, methanol was used instead of PBS solution to dilute NaSa.

The instrument repeatability was tested in order to evaluate if a change in volume of the standard solutions used could influence the calibration curve. Three calibration curves of 0.5ml of NaSa PBS were created and other three using 1ml of the same standard solutions.

2.8.3.2 First Experiment: Existing pyrrole solution in PBS

10 PPySa electrodes coated by Potentiostatic method (N=5) and with Cyclic Voltammetry (N=5) were stored in 0.5ml of PBS solution. PBS was used in order to simulate the body environment. The wires were stored in the oven at 37°C to simulate body temperature. Wires were transferred from the glass vial to another one containing fresh PBS, which was then store again in the oven, with the old vial being retained in the freezer for subsequent analysis by UV. The time points chosen for that experiment to measure the drug release were 10 minutes, 40 minutes, 100 minutes, 1 day, 4 days, 10 days, 18 days and 28 days (see table 2.3).

NaSa concentration has been measured at 296.5 nm wavelength of light absorption in the 200-400nm range using UV 2401 PC UV VIS Recording Spectrophotometer.

2.8.3.3 Second Experiment: Fresh pyrrole solution in PBS

10 PPySa electrodes, coated with the Potentiostatic method (N=5) and with Cyclic Voltammetry (N=5) were stored in 1ml of PBS solution. The method of the experiment is the same as described in section 2.8.3.2.

2.8.3.4 Third Experiment: Fresh pyrrole solution in Methanol

10 PPySa electrodes, coated with the Potentiostatic method (N=5) and with Cyclic Voltammetry (N=5) were stored in 1ml of Methanol. The wires were stored at room temperature. The method of the experiment is the same as described in section 2.8.3.2. Since conducting polymers have been seen to act as antioxidants (Gizdavic-Nikolaidis, 2004), this could have important implications for the use of these polymers as stent coatings, given the potentially negative effect of oxidative stress on outcomes following stenting. The DPPH assay is a common method used to test the ability of polypyrrole to act as a ROS scavenger (Gizdavic-Nikolaidis, 2004). This method is commonly performed in methanol solutions, so the study of drug release in

methanol that was conducted in this project was a preliminary study to determining if the surfaces produced had anti-oxidant capacity and to consider if methanol represented a suitable environment for measuring drug release.

Number of wires	Coating method	Solution	Electropolimerisation duration/ voltage	Storing solution	Storing temperature	Collecting time point	Storing Samples Method
1	Potentiostatic	0.1MNaCl 0.1MPy (existing Py)	5 minutes / 0.9Volts	—	—	—	—
5	Potentiostatic	0.1MNaSa 0.1MPy (existing Py)	5 minutes / 0.9Volts	0.5ml of PBS	37°C in the oven	10 minutes 40 minutes 100 minutes 1 day 4 days 11 days 18 days 28 days	Freezing
5	Cyclic Voltammetry (CV)	0.1MNaSa 0.1MPy (existing Py)	Number of Cycles: 5 Initial Pot: 0V Vertex Pot1: 0.5V Vertex Pot2: 2V Final Pot: 0V	0.5ml of PBS	37°C in the oven	10 minutes 40 minutes 100 minutes 1 day 4 days 10 days 18 days 28 days	Freezing
5	Potentiostatic	0.1MNaSa 0.1MPy(Fresh Py)	5 minutes / 0.9Volts	1ml of PBS	37°C in the oven	10 minutes 40 minutes 100 minutes 1 day 4 days 11 days 19 days 28 days	Freezing
5	Cyclic Voltammetry (CV)	0.1MNaSa 0.1MPy(Fresh Py)	Number of Cycles: 5 Initial Pot: 0V Vertex Pot1: 0.5V Vertex Pot2: 2V Final Pot: 0V	1ml of PBS	37°C in the oven	10 minutes 40 minutes 100 minutes 1 day 4 days 11 days 19 days 28 days	Freezing
5	Potentiostatic	0.1MNaSa 0.1MPy(Fresh Py)	5 minutes / 0.9Volts	1ml of Methanol	Room temperature	10 minutes 40 minutes 100 minutes 1 day 5 days 11 days 18 days 28 days	Room temperature

Table 2.3: Complete table of the experiments.

2.9 Statistical Analysis

2.9.1 Equipment

EXCEL software was used to create the calibration curve and to calculate the drug release from data collected during UV Spectroscopy.

2.9.2 Methods

The concentration of salicylate released from pyrrole coated wires was calculated every time from the calibration curve of regression equation showed below:

$$y = ax^2 + bx + c \quad (2.1)$$

Where (a) is the gradient, (c) is the intercept, (y) is the absorbance and (x) is the unknown sample concentration. This is a polynomial of second order equation and represents the calibration curve. It is obtained by creating a trend line which passes through the data points on a graph of the calibration curve.

From the equation (2.1) the unknown sample concentration (x) can be calculated, using the simple second order polynomial equation showed below.

$$x = \frac{-b+/-\sqrt{b^2-4ac^*}}{2a} \quad (2.2)$$

An additional step is necessary before solving the equation (2.2). In fact the value (c^*) in the equation is unknown and it can be calculated by manipulating the equation (2.1), as showed below:

$$c^* = c - y \quad (2.3)$$

The second order polynomial equation produces two results, depending on the sign chosen before the square root. In the experiment it was calculated only the positive sign because it was necessary to have positive results in the end.

The concentration of salicylate obtained from the equation (2.3) is in µmol, so to calculate the mass of salicylate released over the time period can be calculated as showed below:

$$M=CVm_w \quad (2.4)$$

Where M is the mass (µgrams), C is concentration of Salicylate (µmol/L), V is the volume (Litres) and m_w is the molecular weight of salicylate (160.2 µg/µmol) (Bourke, 2011).

The average of the cumulative mass of the 5 wires was calculated for each time point. With those values it was possible to produce a cumulative drug release versus time graph.

Standard deviation and Standard Error of the Mean (SEM) were calculated in order to provide simple measures of uncertainty in the values. The equation (2.5) used to calculate SEM is showed below:

$$\text{SEM} = \frac{StDev}{\sqrt{(n)}} \tag{2.5}$$

Where StDev is the Standard Deviation and (n) is the number of observations, which in all the experiments is equal to 5.

Percentage of the average of the concentrations was also calculated, to allow the creation of the graph (drug release percentage versus time).

Unless otherwise stated, all data are reported as the average value of 5 replicate samples ± SEM.

CHAPTER 3. RESULTS

3.1 Introduction

In the first Chapter, the drug eluting stent concept was introduced and some of the limitations of existing devices were discussed. The aim of this work was to investigate the development of a novel conducting polymer coating that may overcome some of these limitations, and which may therefore serve as a future drug-eluting stent coating. The methodology used for the experimental aspects of this project has been described in Chapter 2. A series of polypyrrole coatings were produced by electropolymerisation using different experimental conditions. The surfaces of the resultant coatings were then characterised using scanning electron microscopy (SEM) and the drug release kinetics quantified using UV-spectroscopy. The key results of this work are presented in the current chapter.

3.2 Polypyrrole coatings

3.2.1 Potentiostatic method

The electrodes were immersed in a 40ml solution contained in a beaker at room temperature, as described in the Chapter 2. In all experiments, the potentiostatic electropolymerisation was carried out at a fixed potential of 0.9V versus the reference electrode (KR5), for 5 minutes.

Three different types of solution were used in this experiment to coat the stainless steel wires: 0.1M NaCl 0.1M Py solution, 0.1M NaSa 0.1Py (existing Py) solution and 0.1M NaSa 0.1M Py (fresh Py) solution.

During the electropolymerisation, graphs of current density (Amps/cm^2) versus time (sec) were created automatically by the Galvanostat software CorrWare. Although the method of electropolymerisation was the same for every coating, the graphs were observed to be different, according to the different solutions used for the electropolymerisation.

3.2.1.1 NaCl coating

The Potentiostatic electropolymerisation of the wire in 0.1M NaCl 0.1M (existing py) solution was successfully. The wire showed a thick black coating. The figure below presents the current density (Amps/cm^2) versus time (sec) observed for the coatings using NaCl as the dopant ion (Figure 3.1). Only one wire was coated with NaCl, as just a preliminary work to establish that the coating could be produced successfully using a well-studied and characterised dopant ion such as NaCl.

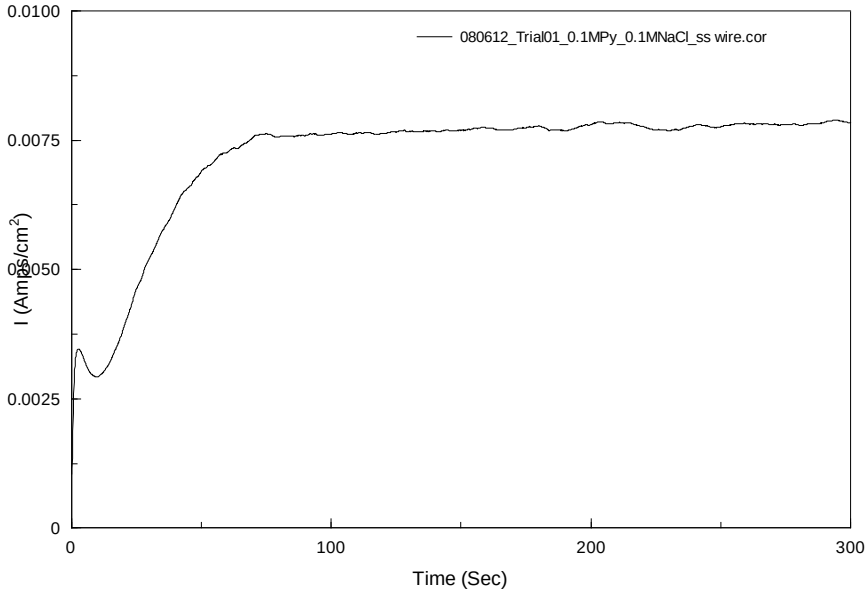


Figure 3.1: Potentiostatic Current Density (Amp/$\mathbf{cm^2}$) versus Time (Sec) graph of SS wire in 0.1M NaCl 0.1M Py solution at 0.9V for 5 minutes. Graph produced with CorrWare.

In the figure 3.1, it can be noticed that an increase in time corresponds with an increase in current density until the curve maintains a constant behaviour at a value of around 0.0075 Amps/cm^2.

3.2.1.2 NaSa coating (existing Pyrrole)

In this experiment, 5 wires were coated potentiostatically in 0.1M NaSa 0.1M Py solution. Pyrrole used for this experiment was from an existing bottle that had been stored at 4°C for approximately three months under inert gas. After electropolymerisation, the wires showed a light black coating, which was not

homogeneous and it was easy to see the stainless steel colour through the coating. Figure 3.2 shows the graph of current density (Amps/cm^2) versus time (sec) for the coatings. Although the procedure and the solution was the same for every coating, the current-time profile observed for each coating can be seen to be slightly different to each other. From figure 3.2, it can be seen that in the first 30 sec after the start of applying the potential, current decreases until it stabilises at a certain current value. This behaviour is in contrast to the profile produced during the NaCl coating (Figure 3.1).

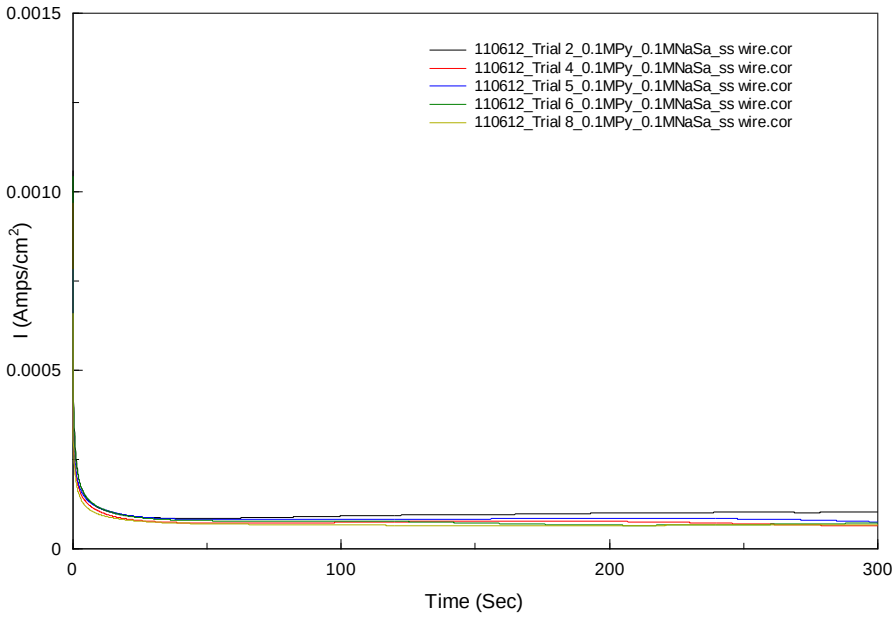


Figure 3.2: Potentiostatic Current Density (Amp/$\boldsymbol{cm^2}$) versus Time (sec) graph of 5 SS wires in 0.1M NaSa 0.1M (existing Py) solution at the potential of 0.9V for 5 minutes.

3.2.1.3 NaSa coating (fresh Pyrrole)

On inspection of the pyrrole solution used in the above experiments, it was found that it was considerably darker in appearance than a fresh bottle of pyrrole solution that had yet to be opened. This observation, combined with the unexpected current-time profiles observed during the above coatings with NaSa, meant that a decision was taken to repeat the above experiment using the fresh pyrrole solution. In this experiment, 5 wires were coated with 0.1M NaSa 0.1M Py solution. The coating

produced showed a more uniform coating compared to the previous coatings. The figure below shows the graph current density (Amps/ cm^2) versus time (sec) generated for these coatings (figure 3.3).

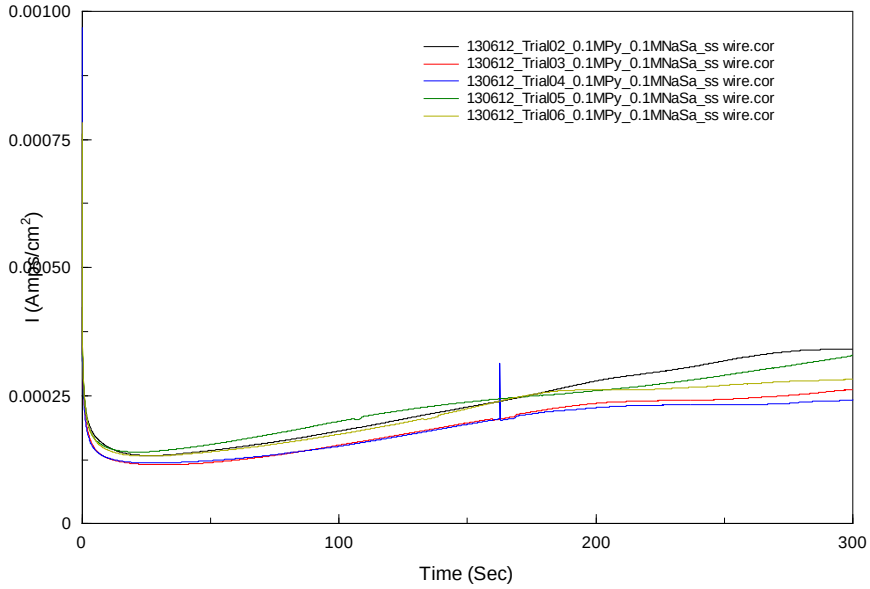


Figure 3.3: Potentiostatic Current Density (Amp/$\boldsymbol{cm^2}$) versus Time (sec) graph of 5 SS wires in 0.1M NaSa 0.1M (fresh py) solution at the potential of 0.9V for 5 minutes.

3.2.1.4 NaCl/NaSa coatings comparison

To have a better understanding of the differences between the three different types of coating, the NaCl/NaSa coatings output have been combined in the same graph (see figure 3.4). It can be seen that the three different coatings showed three completely different profiles. In the NaCl output there is an increase in current density over time, which is still present in the NaSa (fresh py) output, although smaller compared to the NaCl one. On the contrary, the NaSa (existing py) output seemed to not have this increase in current density (see figure 3.4).

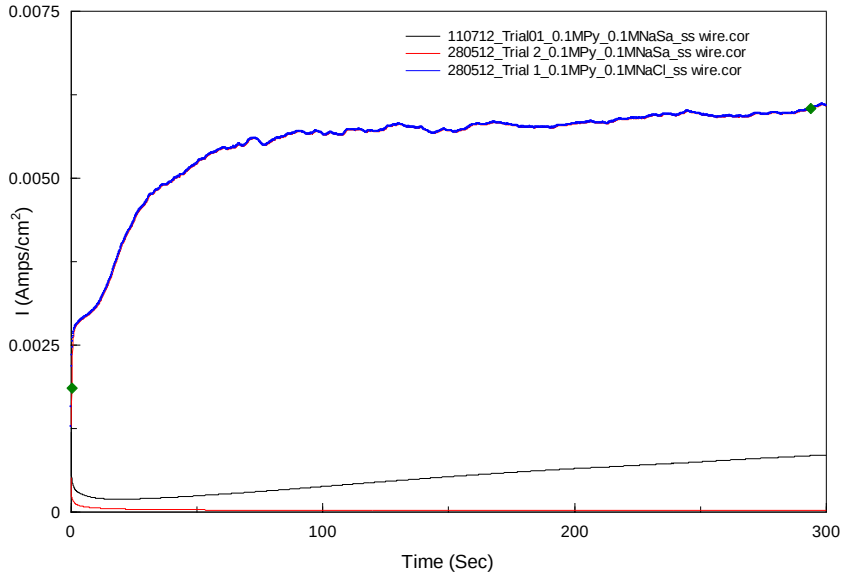


Figure 3.4: Comparison of 3 Potentiostatic Current density versus Time (sec) graphs of ss wires in: (blue) 0.1M NaCl 0.1M (existing py) solution; (red) 0.1M NaSa 0.1M (existing py) solution; (black) 0.1M NaSa 0.1M (fresh py) solution.

In figure 3.5, the outputs of the two NaSa coatings, one coated with existing Py solution, the other one with fresh one. It can be seen that the two profiles seemed to be different.

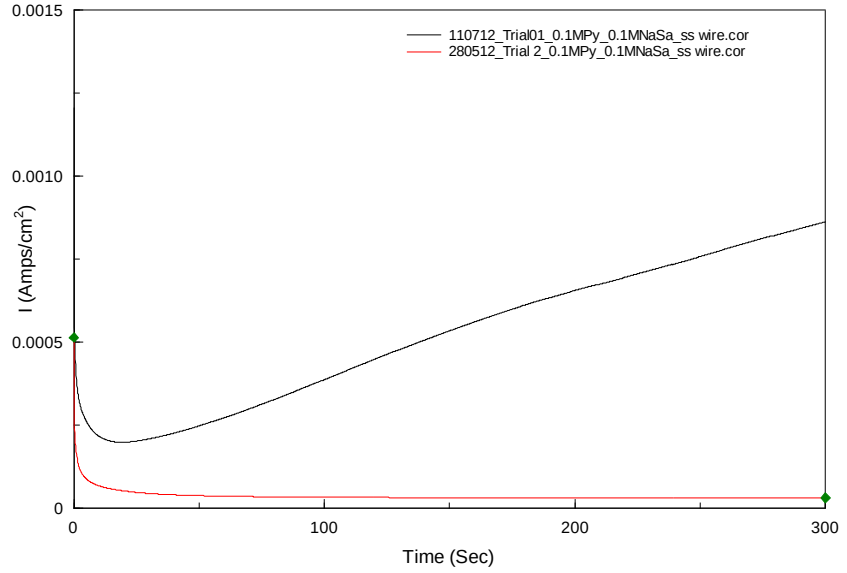


Figure 3.5: Comparison of 2 Potentiostatic Current density versus Time (sec) graphs of ss wires in: (red) 0.1M NaSa 0.1M (existing py) solution; (black) 0.1M NaSa 0.1M (fresh py) solution.

3.2.2 Cyclic Voltammetry method

The wires coated through cyclic voltammetry electropolymerisation showed a thick black coating. The electrodes were immersed in a 40ml solution contained in a beaker at room temperature as described in the Chapter 2. The Cyclic Voltammetry electropolymerisation was carried out for every coating at the vertex potential 1 of 0.5V and vertex potential 2 of 2V for 5 cycles, when the initial and the final potential were set at 0V.

Two different types of solution have been used in this experiment to coat the wires, 0.1MNaSa 0.1Py (existing Py) solution and 0.1MNaSa 0.1MPy (fresh Py) solution.

During the electropolymerisation graphs of current density (Amps/cm^2) versus potential difference (Volt) were created automatically by the Galvanostat software CoreView. Although the procedure was the same for every coating, graphs can be seen to be different, according to the different solution used for the electropolymerisation.

3.2.2.1 NaSa coating (existing Py solution)

In this experiment, 5 wires were coated with 0.1M NaSa 0.1M Py solution. Pyrrole used to make the solution was taken from an old batch of Pyrrole. The figure shows the graph current density (Amps/cm^2) versus potential different (Volt) of the coatings.

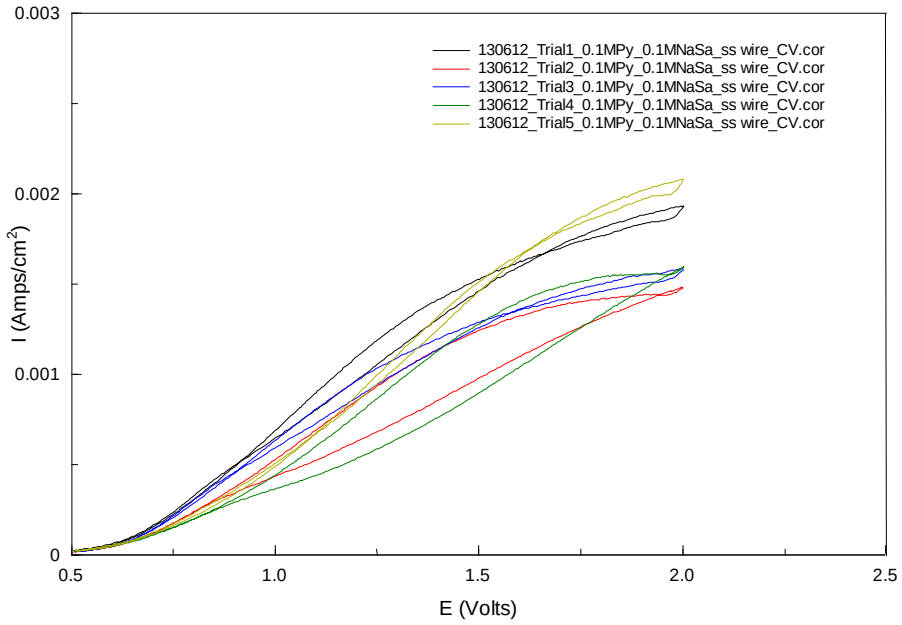


Figure 3.6: Cyclic Voltammetry Current density versus Time (sec) graph of 5 ss wires in 0.1M NaSa 0.1M (existing py) solution at the range of 0.5V-2V for 5 cycles.

3.2.2.2 NaSa coating (fresh Pyrrole)

In this experiment, 5 wires were coated with 0.1M NaSa 0.1M Py solution. Pyrrole used to make the solution was taken from a new batch of Pyrrole. The figure 3.6 shows the graph of current density (Amps/cm^2) versus potential different (Volt) of the coatings.

From this picture it can be seen that even for the Cyclic Voltammetry polymerisation, the output of the coating with fresh Py solution is different from the output of the existing Py solution.

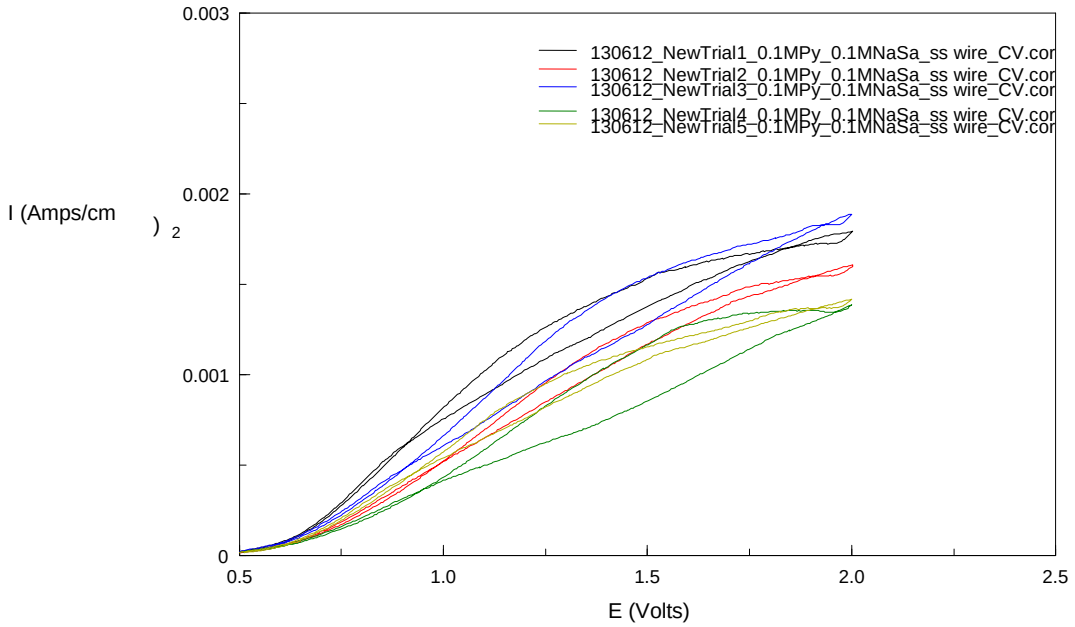


Figure 3.7: Cyclic Voltammetry Current density versus Time (sec) graphs of 5 ss wires in 0.1M NaSa 0.1M (fresh py) solution at the range of 0.5V-2V for 5 cycles.

3.2.3 Charge calculation

The charge applied to the electrodes during electropolymerisation was calculated using the Potentiostat/Galvanostat software CoreView. Charge values of each wire are showed in the table below.

Number of Wires	Electropolimerisation Method	Electropolymerisation Solution	Buffer Solution	Charge Average
5	Potentiostatic	0.1NaSa 0.1(existing Py)	PBS	0.006632 ± 0.000229
5	Cyclic Voltammetry	0.1NaSa 0.1(existing Py)	PBS	0.097129± 0.003772
5	Potentiostatic	0.1NaSa 0.1(fresh Py)	PBS	0.009829± 0.0004
5	Cyclic Voltammetry	0.1NaSa 0.1(fresh Py)	PBS	0.084204± 0.006001

5	Cyclic Voltammetry	0.1NaSa 0.1(fresh Py)	Methanol	0.0842792 ± 0.003908
5	Potentiostatic	0.1NaSa 0.1(fresh Py)	Methanol	0.00943± 0.000998

Table 3.1: Table of the wires used in the experiments and their charge values**.**

3.3 SEM Analyses

As described in Chapter 2 (section 2.7.2), SEM analyses was performed to investigate the characteristics of the coating surface.

Images of the surface and the cross section area of each type of coating are shown in this section.

3.3.1 Potentiostatic NaCl coating

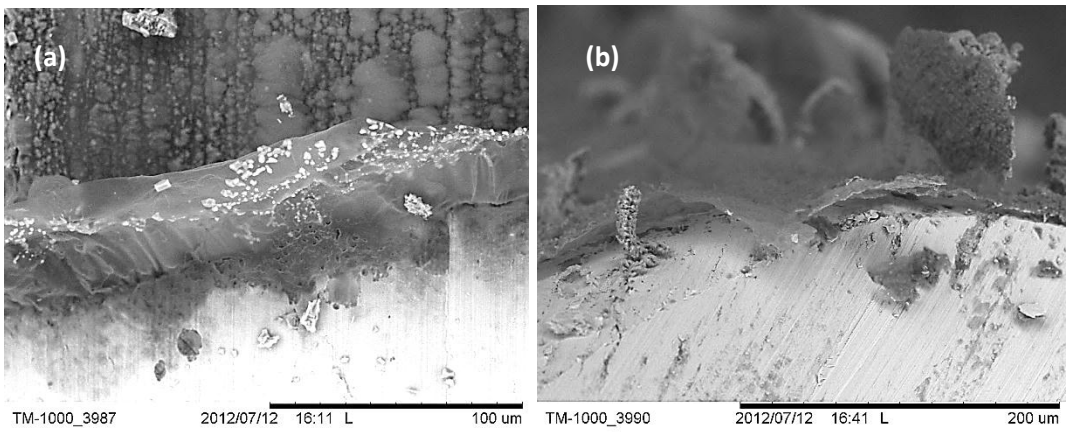


Figure 3.8: Two different SEM images of a ss wire coated through potentiostatic electropolymerisation in 0.1M NaCl 0.1M (existing py) solution. (a) Surface section of the wire where it is visible the boundary between the coating (dark part) and the stainless steel wire (light part). (b) Cross-section area of the wire where it is visible the stainless steel wire with the external coating.

From the SEM images (figure 3.8) it can be seen that a polymer coating is produced, with a clear boundary between the coated and non-coated sections (figure 3.8 (a)). The coating surface is not uniform and presents a significant level of roughness, as it can be seen clearly on the cross section area of the coating (figure 3.8 (b)).

3.3.2 Potentiostatic NaSa coating (existing Py)

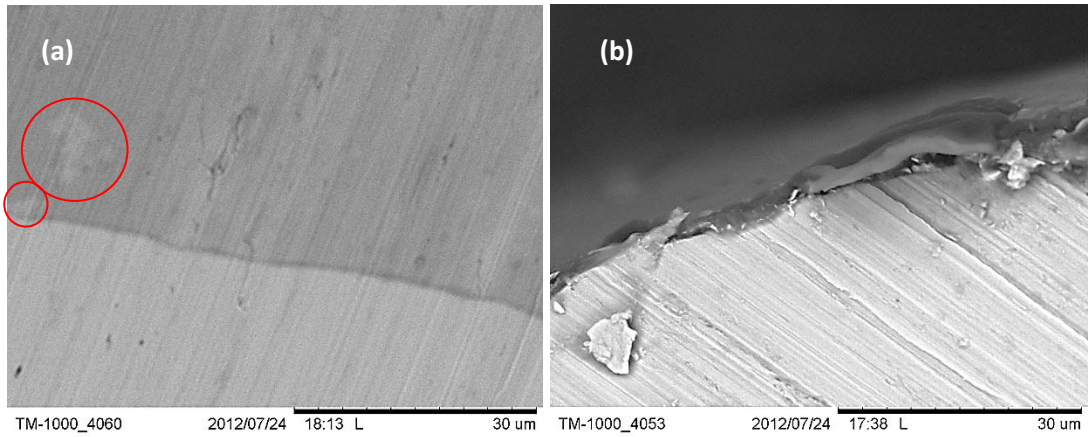


Figure 3.9: Two different SEM images of a ss wire coated through potentiostatic electropolymerisation in 0.1M NaSa 0.1M (existing py) solution. (a) Surface section of the wire where it is visible the boundary between the coating (dark part) and the stainless steel wire (light). Non-coated areas of the surface have been highlighted. (b) Cross-section area of the wire where it is visible the stainless steel wire with the external coating.

In the SEM figures 3.9 showed above, it can be seen that the surface of the NaSa coating is very smooth and thin, and presents some non-coated areas on the surface (figure 3.9(a)). It was difficult to take the SEM image of the cross section area of this wire where the coating was visible. This was because of the very thin coating of this wire.

3.3.3 Potentiostatic NaSa coating (fresh Py)

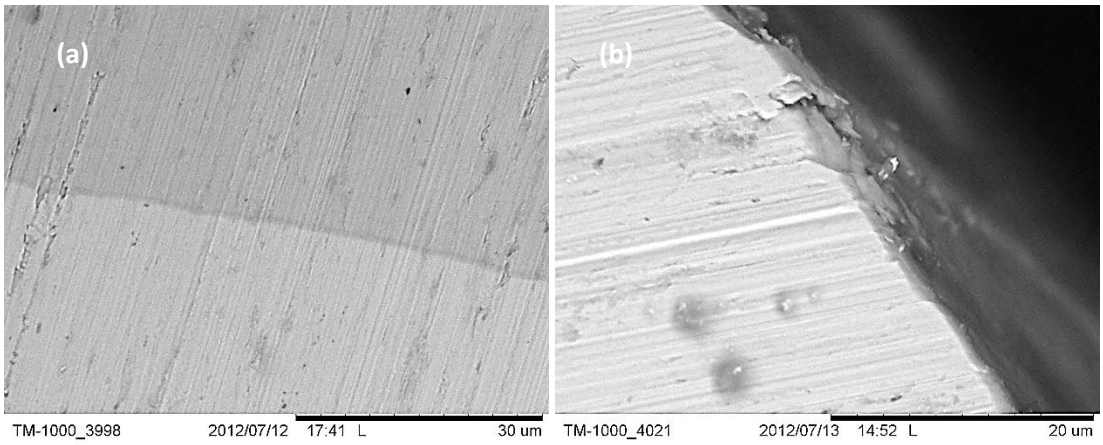


Figure 3.10: Two different SEM images of a ss wire coated through potentiostatic electropolymerisation in 0.1M NaSa 0.1M (fresh py) solution. (a) Surface section of the wire where it is visible the boundary between the coating (dark part) and the stainless steel wire (light part). (b) Cross-section area of the wire where it is visible the stainless steel wire with the external coating.

In the SEM images showed above (figure 3.10 (a)), it can been seen that the coating with fresh Py presents a smooth and more homogeneus covering of the wire, compared to the NaSa coating with existing Py solution (figure 3.9 (a)). Even in this case, it was very difficult to take the SEM image of the cross section area of this wire where the coating was visible. This was because of the very thin coating of this wire.

3.3.4 Cyclic Voltammetry NaSa coating (existing Py)

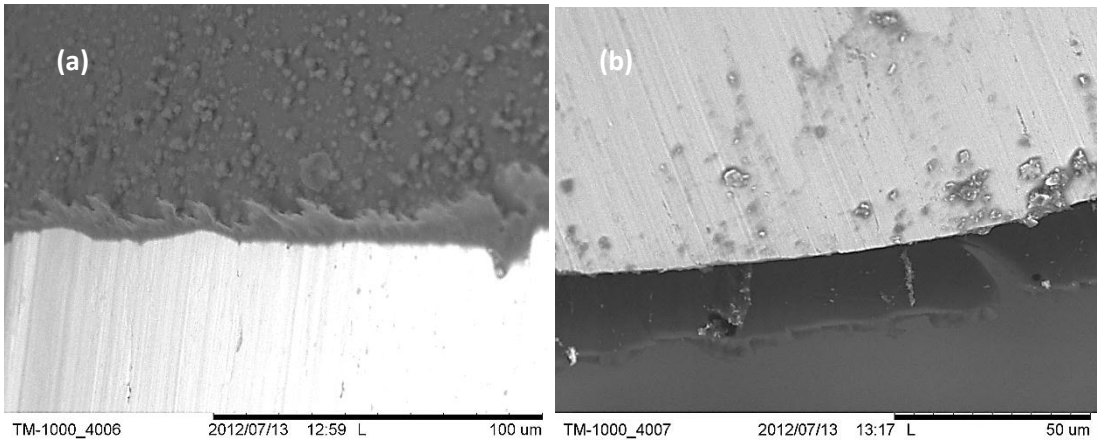


Figure 3.11: Two different SEM images of a ss wire coated through Cyclic Voltammetry electropolymerisation in 0.1M NaSa 0.1M (existing py) solution. (a) Surface section of the wire where it is visible the boundary between the coating (dark part) and the stainless steel wire (light part). (b) Cross-section area of the wire where it is visible the stainless steel wire with the external coating.

In the SEM images above (figure 3.11 (a)), it can be seen that the surface of the NaSa coating of CV method presents relatively large particles, and at higher magnification it is possible to see its consistency and significant thickness (figure 3.11 (b)).

3.3.5 Cyclic Voltammetry NaSa coating (fresh Py)

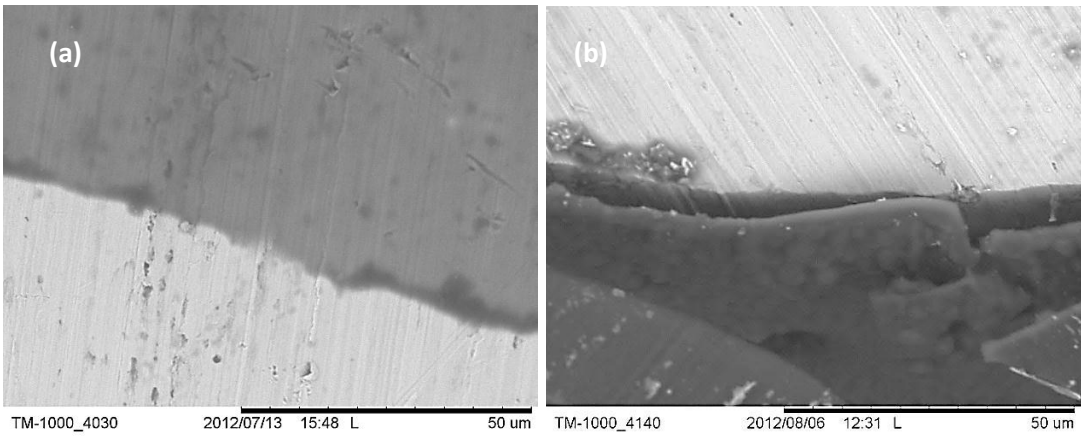
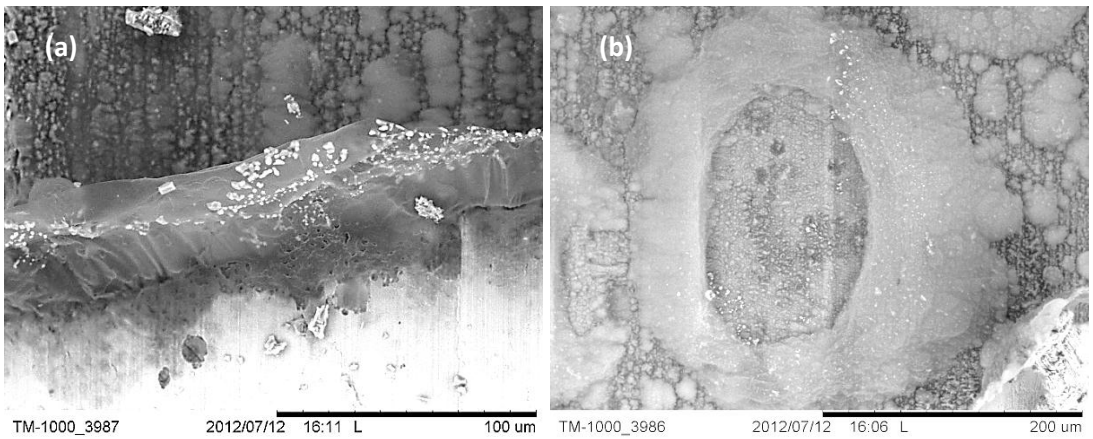


Figure 3.12: Two different SEM images of a ss wire coated through Cyclic Voltammetry electropolymerisation in 0.1M NaSa 0.1M (fresh py) solution. (a) Surface section of the wire where it is visible the boundary between the coating (darker part) and the stainless steel wire. (b) Cross-section area of the wire where it is visible the stainless steel wire with the external coating.

Cyclic Voltammetry NaSa coating with fresh Py solution showed a homogeneus covering of the wire (figure 3.12 (a)). The SEM image of the cross section area of this coating shows a thick, dark coating with large particles on the surface (figure 3.12 (b)).

3.3.6 Potentiostatic/ Cyclic Voltammetry coatings SEM images comparison

To allow comparison of the different coatings, SEM images of the surface of each coating are showed below.



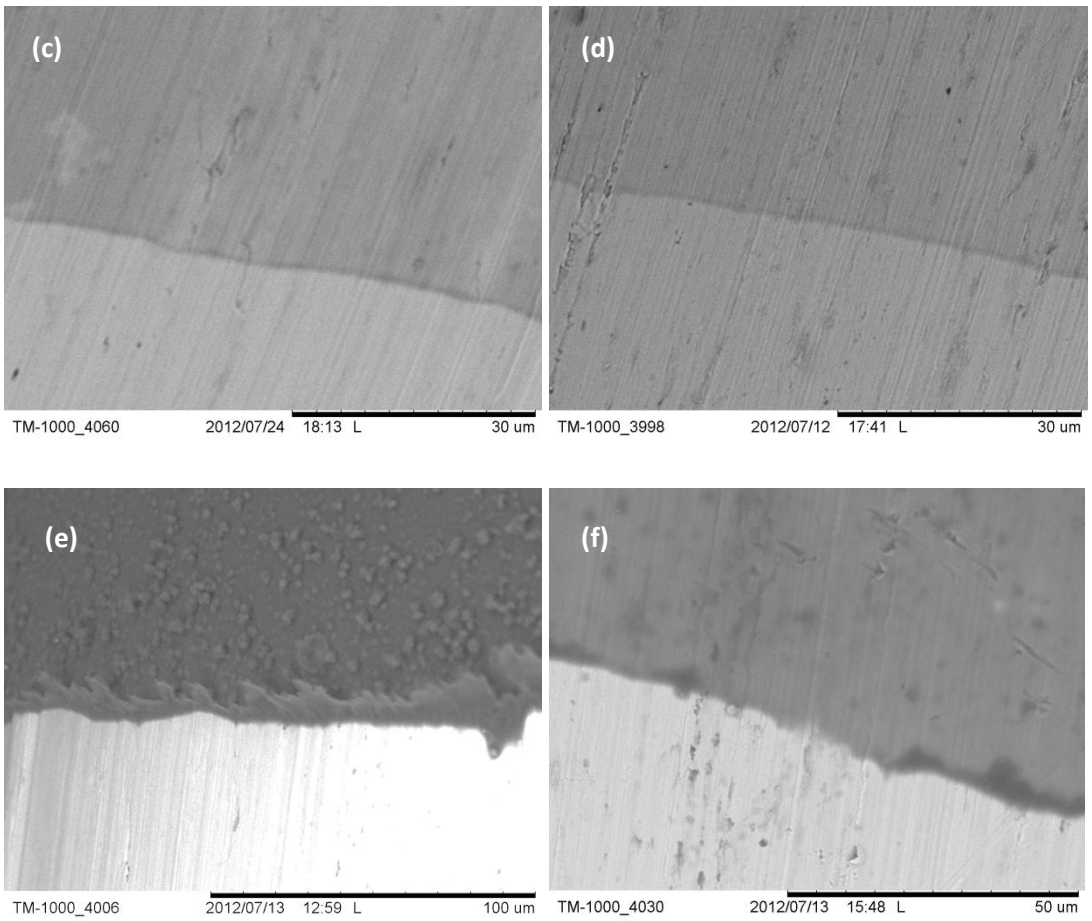


Figure 3.13: Comparison of the SEM images of Potentiostatic and Cyclic Voltammetry coatings. The SEM images show the surface section of the wires where it is visible the boundary between the coating (dark part) and the stainless steel wire (light part) of the all different coatings: **(a)** Potentiostatic NaCl and (existing py) coating; **(b)** particular of the Potentiostatic NaCl and (existing py) coating; **(c)** Potentiostatic NaSa (existing py) coating; **(d)** Potentiostatic NaSa (fresh py) coating; **(e)** Cyclic Voltammetry NaSa (existing py) coating; **(f)** Cyclic Voltammetry NaSa (fresh py) coating.

In the SEM images showed above (figure 3.13), it is clear that different methodologies of coating correspond to different characteristics of the surface of the wires.

Potentiostatic coating with NaSa (fresh Py) solution showed a smoother and more homogenous surface (figure 3.13(d)), compared to the wire coated with the NaSa (existing Py) solution (figure 3.13(c)). Important differences between the two different CV coatings can be seen in the figure 3.13. The coating produced with fresh py solution (figure 3.13(f)) presents a smoother and thinner surface with fewer particles. The colour of the coating is lighter and in this image (figure 3.13(f)) the

particles on the surface are so small that they are not visible, compared to the coating produced with existing py.

3.4 Drug release measurement

After having collected all the samples for each time point of every experiment, drug release concentration can be measured by performing the UV/VIS spectrometry as describe in the section 2.8.3. Before proceeding to analyse the concentration of the samples, a calibration curve needs to be made by measuring a series of standard concentrations of the specific drug.

3.4.1 UV/VIS spectrophotometer repeatability

Six Calibration curves were created to evaluate the repeatability of the instrument in case the volume used in the cuvettes is different as described in the section 2.8.3.1. Three calibration curves were produced using 0.5ml of NaSa standards during the UV/VIS spectrometry. Another three calibration curves were produces using 1ml of NaSa standards, in order to evaluate if the calibration curves obtained with different volumes are similar or different.

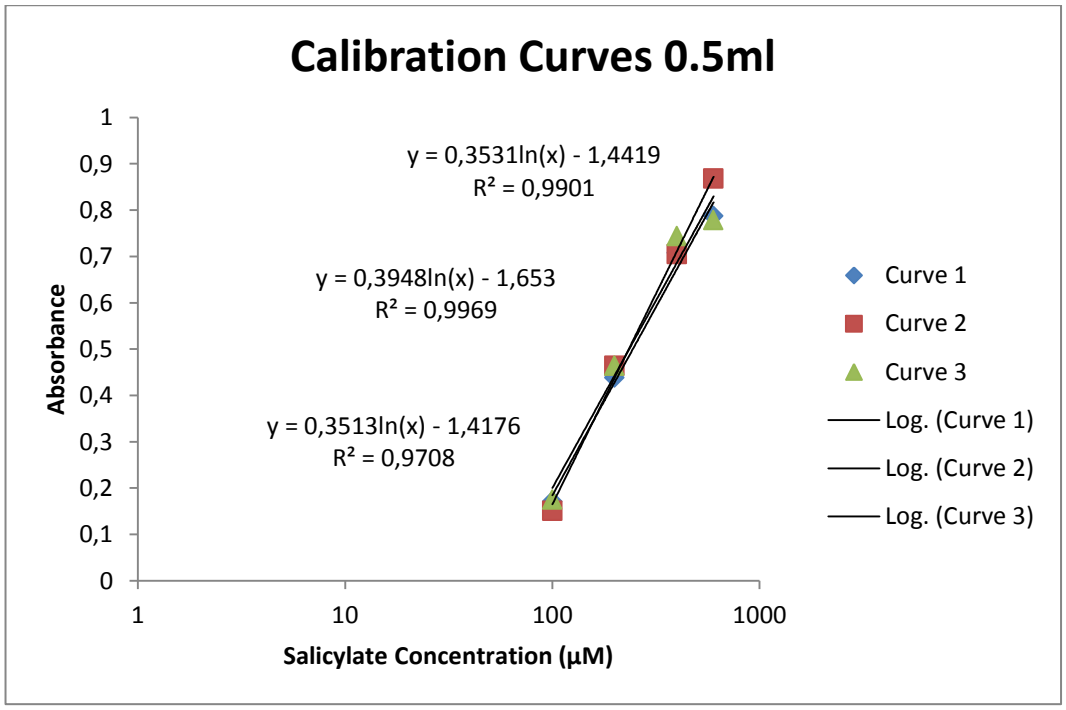


Figure 3.14: Comparison of 3 Calibration curves produced using 0.5ml of NaSa standards in the concentration range of 100 - 600µM.

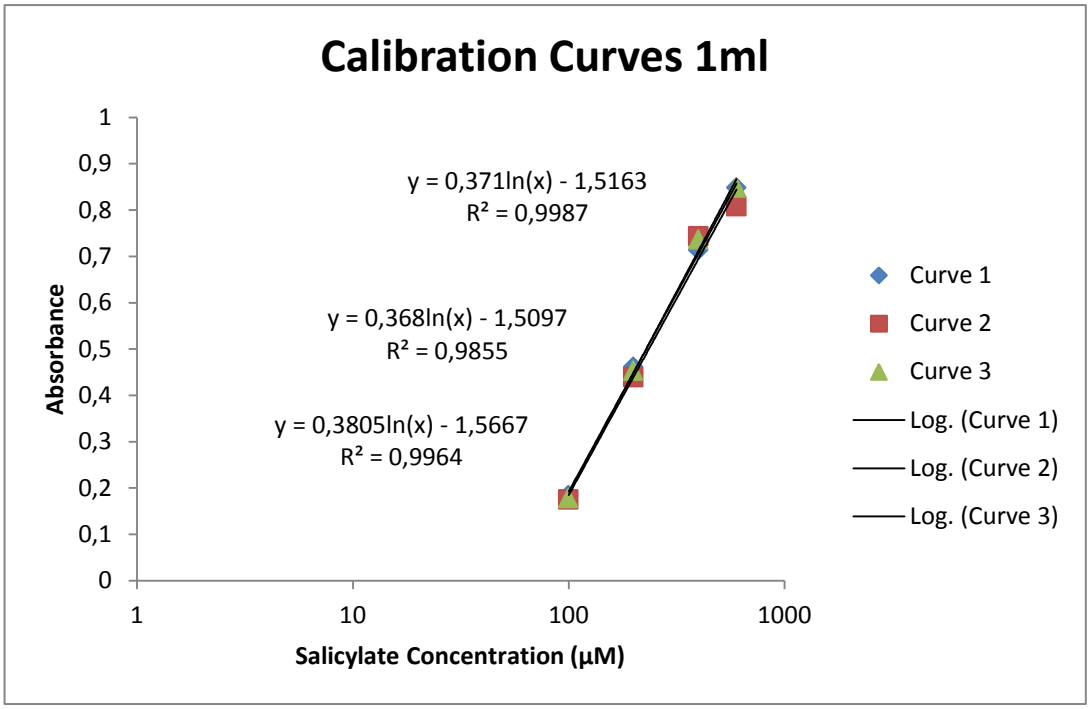


Figure 3.15: Comparison of 3 Calibration curves produced using 1ml of NaSa standards in the concentration range of 100 - 600µM.

In both figures (3.14 and 3.15) the three calibration curves are almost overlapping, so the results are reliable and the repeatability of the instrument is satisfactory.

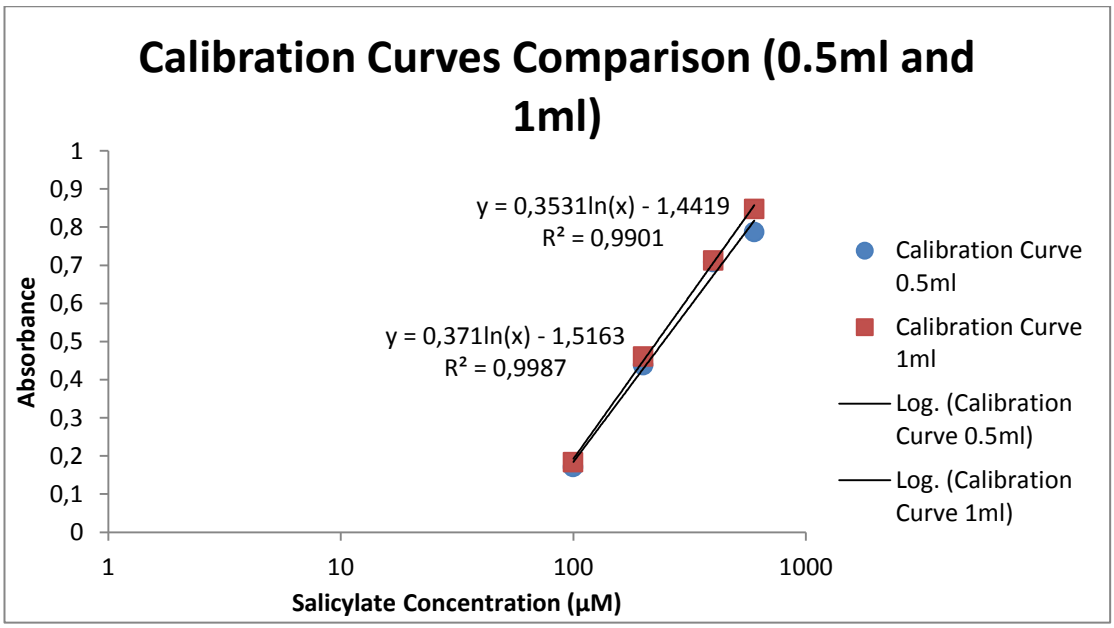


Figure 3.16: Comparison of 2 Calibration curves produced using 0.5ml and 1ml of NaSa standards in the concentration range of 100 - 600µM.

In the figure (3.16), it can be seen that the calibration curves of 0.5ml and 1ml of solution in the concentration range of 100 - 600µM are almost overlapping, so the changing in volume of the standards did not influence the calibration curve.

3.4.2 First drug release measurement: CV/Pot coatings (existing Py) in PBS

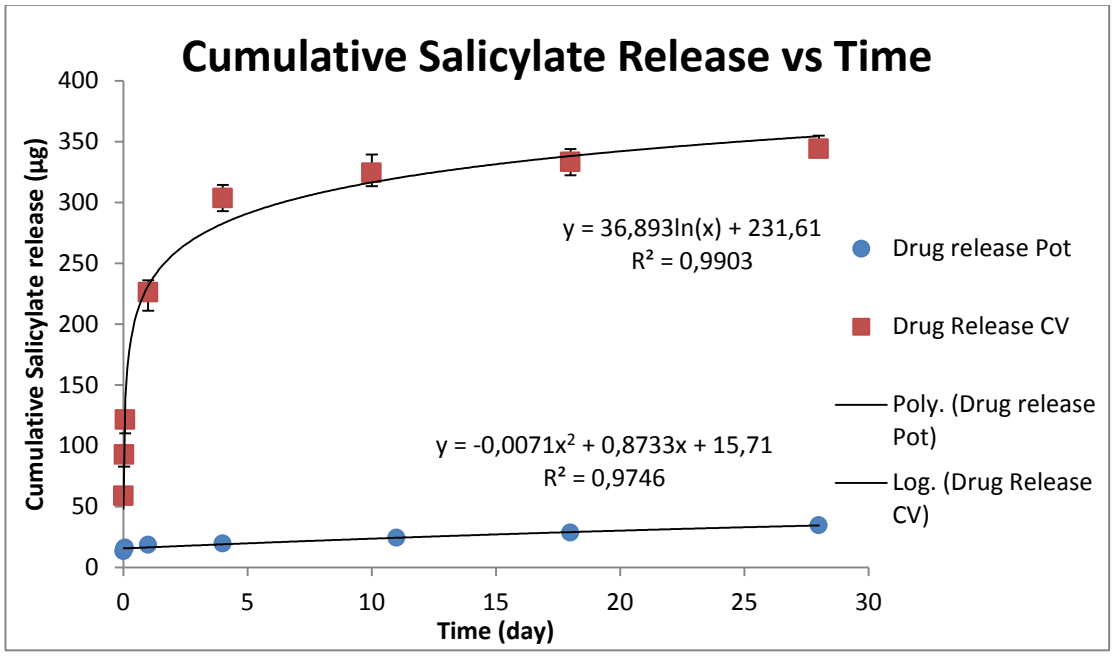


Figure 3.17: Comparison of Cumulative Salicylate release in PBS versus Time profiles of the 5 wires coated through potentiostatic method and the 5 wires coated through CV method in 0.1M NaSa 0.1M (existing py) solution.

In figure 3.17 it can be seen that the drug release profile is different between CV and Potentiostatic coatings. After 28 days approximately, almost 350µg of salicylate were released from the CV coatings in PBS solution. This compares to 25µg from Potentiostatic coatings at the same time point.

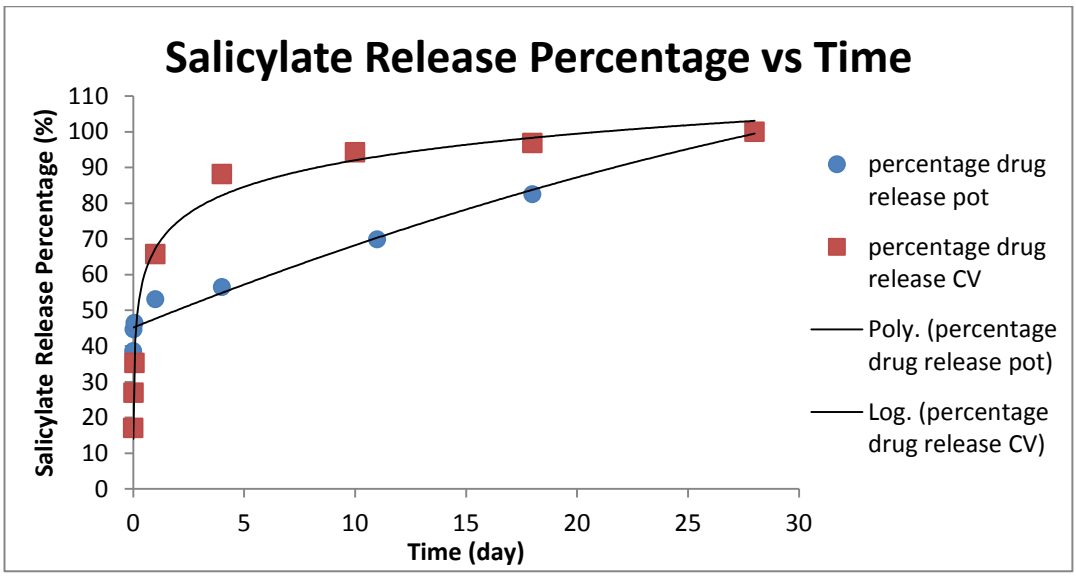


Figure 3-18: Comparison of Salicylate release in PBS percentage versus Time profiles of the 5 wires coated through potentiostatic method and the 5 wires coated through CV method in 0.1M NaSa 0.1M (existing py) solution.

In the figure (3.18), it can be seen that both coatings presented an initial burst. It corresponds to 35% of salicylate release for the CV coatings and almost 50% for the Potentiostatic ones in the first hour. After four days, the CV coatings are seen to have released the 90% of salicylate, compared to the 56% of the Potentiostatic ones.

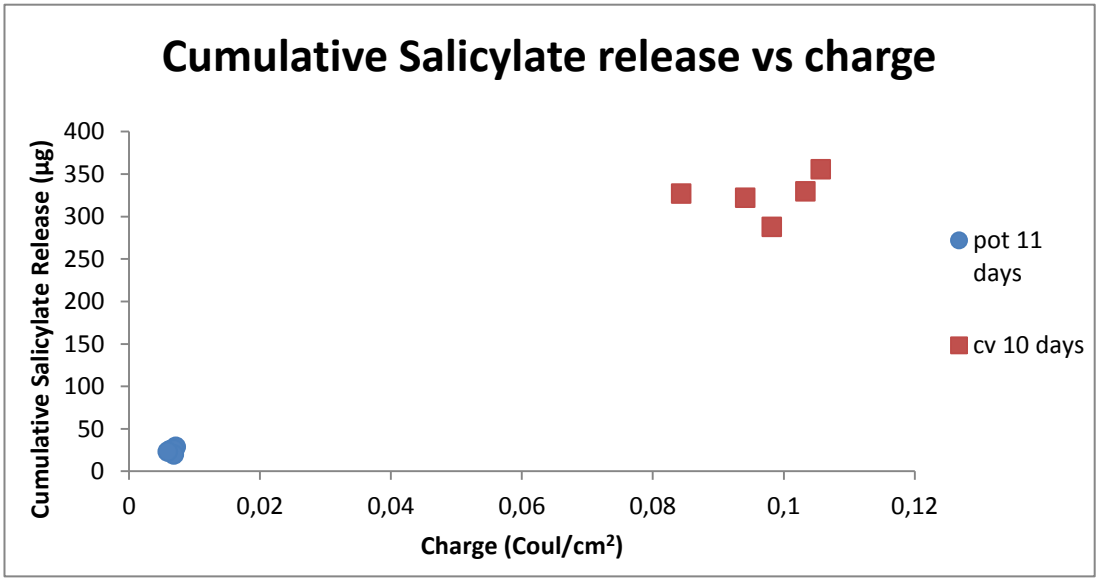


Figure 3.19: Comparison of Cumulative Salicylate release in PBS versus Charge profiles of the 5 wires coated through potentiostatic method and the 5 wires coated through CV method in 0.1M NaSa 0.1M (existing py) solution at 10/11 days of time point.

The relationship between the charge applied to the wires during electropolymerisation and the cumulative salicylate release is showed in the figure (3.17). Values of the charge were taken from the table 3.1. It can be seen that the amount of charge applied during Potentiostatic polymerisation was much small (range of 0.003-0.008 Coul/cm^2), compared to the charge applied during CV polymerisation (range of 0.08-0.11 Coul/cm^2). It is clear from the figure how the cumulative drug release of each coating is directly proportional to the charge applied, consequently at higher charge corresponds a higher drug release.

3.4.3 Second drug release measurement: CV/Pot coatings (fresh py) in PBS

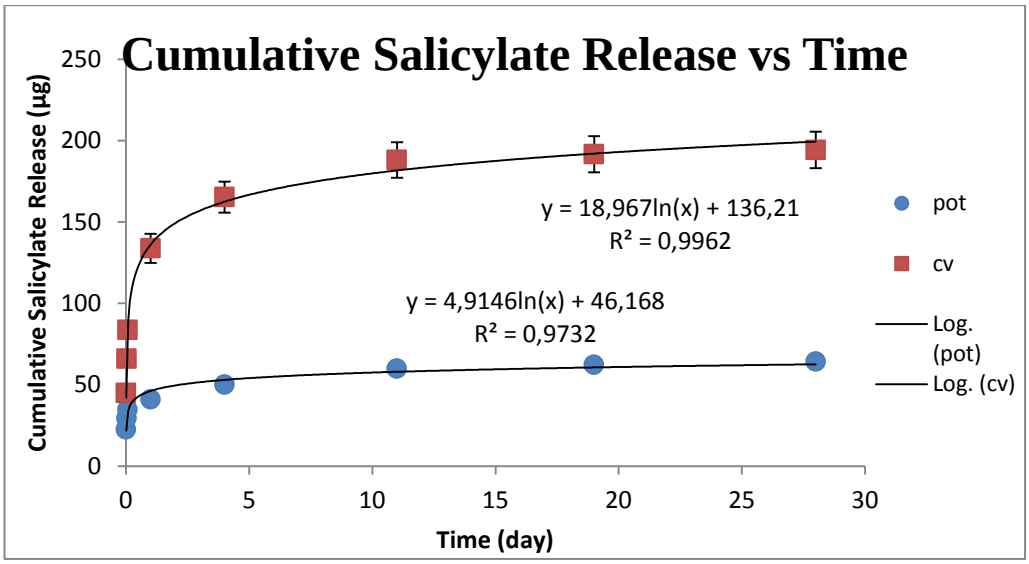


Figure 3.20: Comparison of Cumulative Salicylate release in PBS versus Time profiles of the 5 wires coated through potentiostatic method and the 5 wires coated through CV method in 0.1M NaSa 0.1M (fresh py) solution.

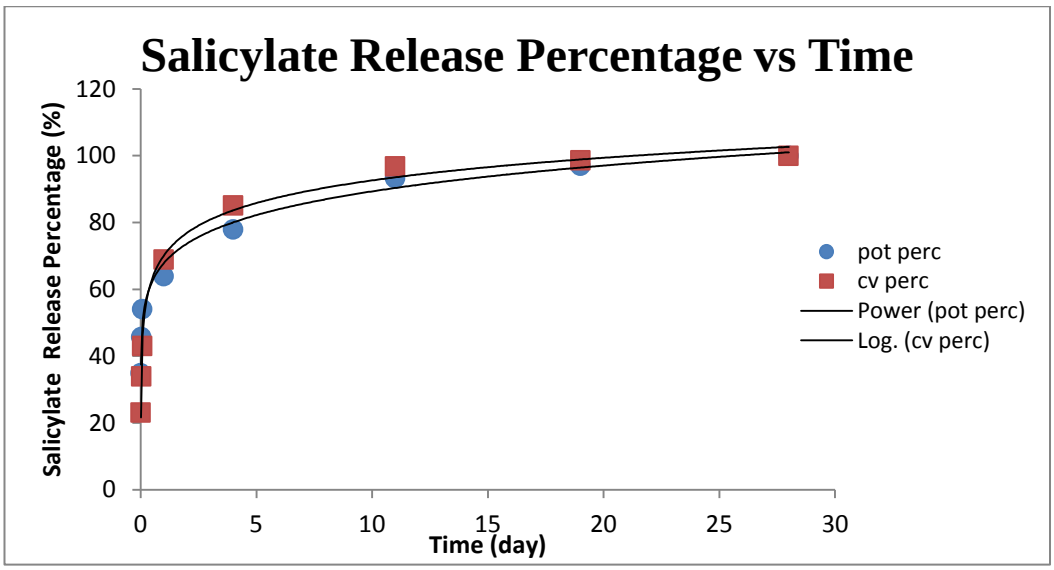


Figure 3.21: Comparison of Salicylate release in PBS percentage versus Time profiles of the 5 wires coated through potentiostatic method and the 5 wires coated through CV method in 0.1M NaSa 0.1M (fresh py) solution.

In the figure (3.21), it can be seen that the drug release profile is different between CV and Potentiostatic coatings. After 28 days, almost 200µg of salicylate were released from the CV coatings and 50µg from Potentiostatic ones.

The salicylate release percentage of the two coatings is seen to be very similar.

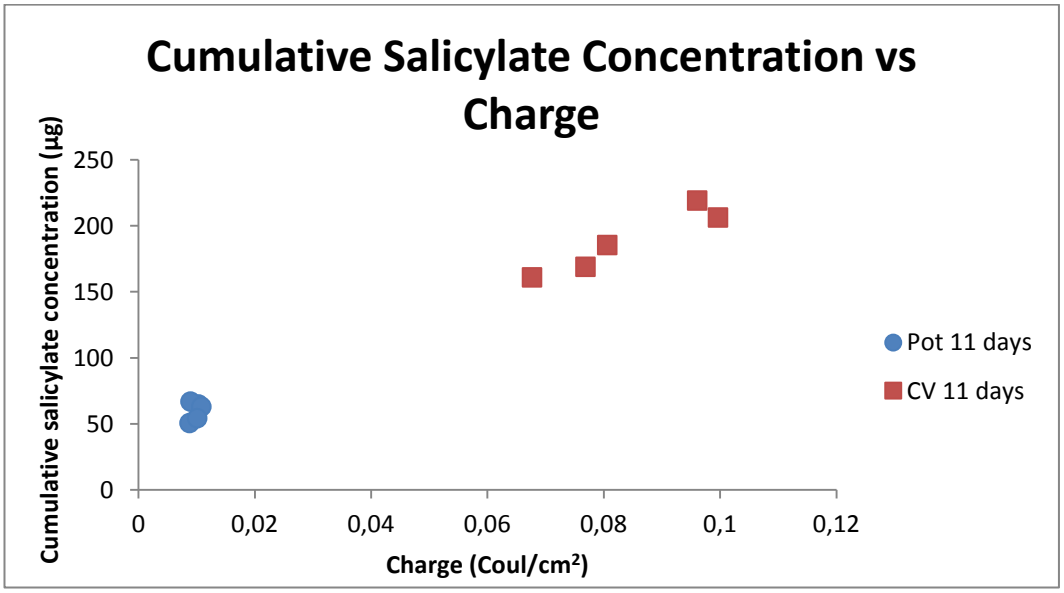


Figure 3.22: Comparison of Cumulative Salicylate release in PBS versus Charge profiles of the 5 wires coated through potentiostatic method and the 5 wires coated through CV method in 0.1M NaSa 0.1M (fresh py) solution at 11 days of time point.

In the figure (3.22) it can be seen that that even in this case, the amount of charge applied during CV electropolymerisation was higher than during the potentiostatic one. At higher charge corresponds again a higher drug release concentration (higher for the CV).

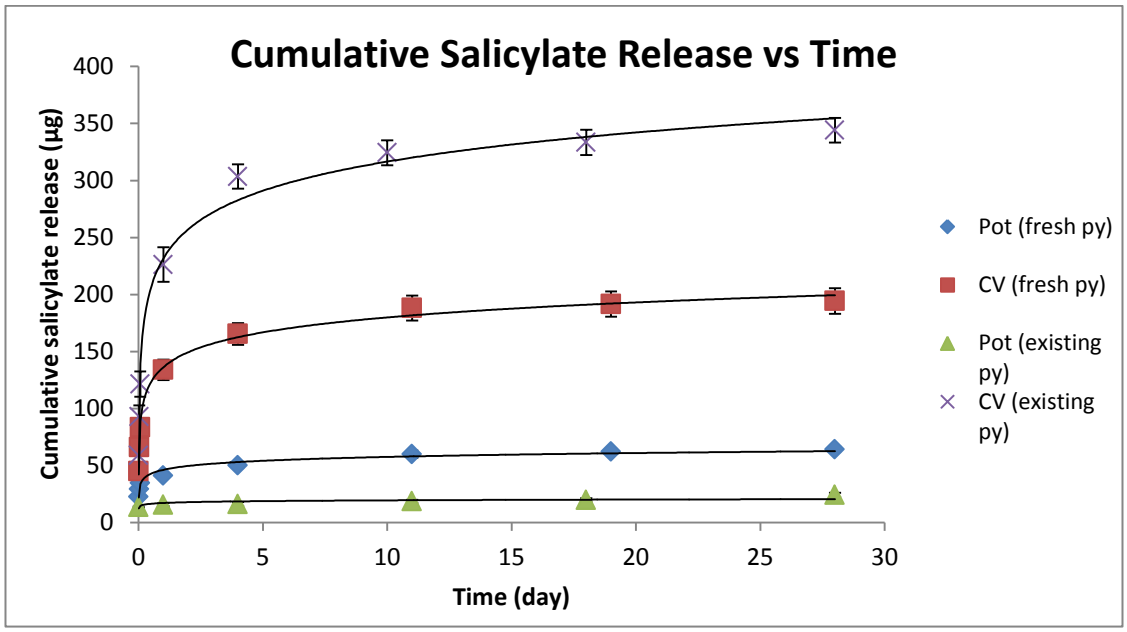


Figure 3.23: Comparison of Cumulative Salicylate release in PBS versus Time profiles of the 4 different coatings in 0.1M NaSa 0.1M (fresh and existing py) solution.

In the figure (3.23) the comparison of the drug release trendlines of the all different coatings can be seen. The wire coated through CV with NaSa (existing py) solution presents the highest drug release profile over time. On the contrary, the wire coated through Pot with NaSa (existing py) solution appeared to have released the lowest amount of drug over time.

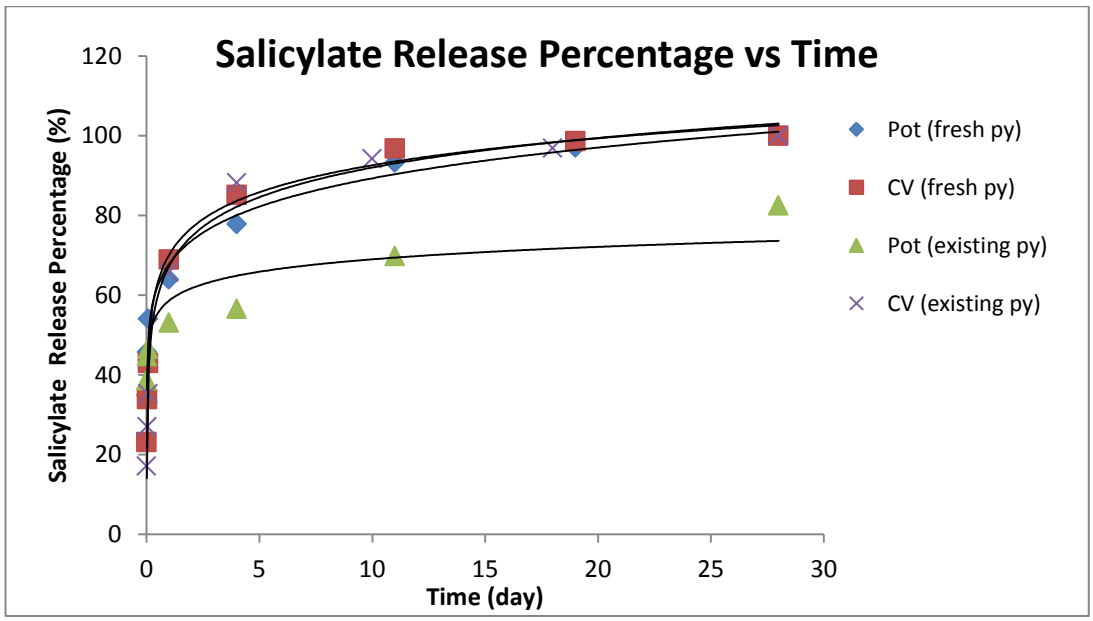


Figure 3.24: Comparison of Salicylate release in PBS percentage versus Time profiles of the 4 different coatings in 0.1M NaSa 0.1M (fresh and existing py) solution.

In the figure (3.24) the drug release percentage trendlines of all the coating are compared. It can be seen that apart from the wire coated through Pot with NaSa (existing py) solution, the other coatings present almost the same drug release percentage profile.

3.4.4 Third drug release measurement: Pot/CV coatings (fresh py) in Methanol

Wires coated through Potentiostatic electropolymerisation and immersed in methanol were shown to have released no drug over a period of 28 days.

The coating charge applied during electropolymerisation was calculated for each wire and compared to the charge of the potentiostatic wires used for the drug release measurement in PBS (see table 3.1). The results showed that the average of the charge of the 5 wires used in methanol were similar to the 5 wires used in PBS. Although the amount of charge applied during electropolymerisation was similar, the results of the drug release measurement showed that the potentiostatic wires did not release any drug in methanol over time, compared to the potentiostatic wires that were seen to have released salicylate over time (see figure 3.20).

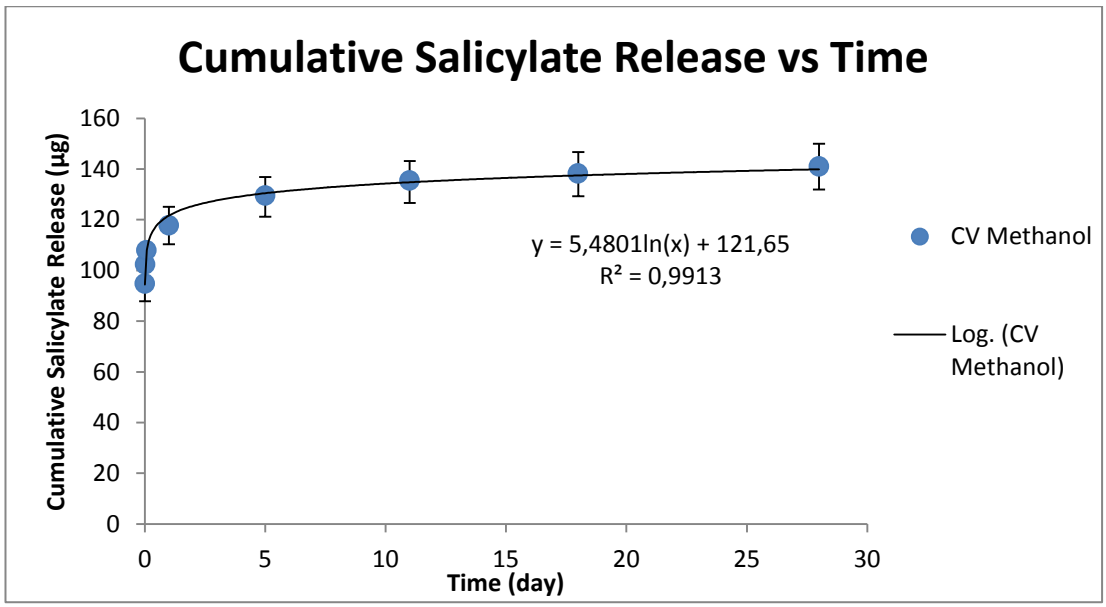


Figure 3.25: Cumulative Salicylate release in Methanol versus Time profile of the 5 wires coated through CV method in 0.1M NaSa 0.1M (fresh py) solution.

In the figure showed above, the cumulative drug release trendline of the wire coated through CV electropolymerisation in Methanol can be seen. At one day time point, the wired released almost 120µg of salicylate, which correspond to the almost 85% of the total drug released over 28 days (figure 3.25).

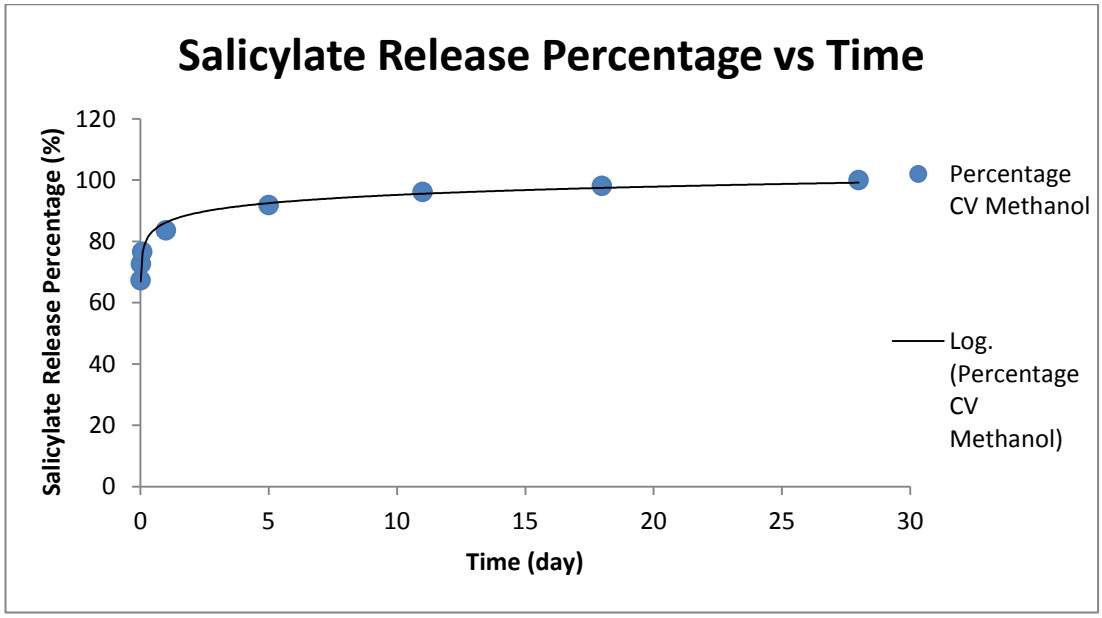


Figure 3.26: Cumulative Salicylate release in Methanol percentage versus Time profile of the 5 wires coated through CV method in 0.1M NaSa 0.1M (fresh py) solution.

3.4.5 Drug release of CV coatings (fresh py) in PBS and Methanol comparison

To understand better the differences of the two different coatings, in the figure (3.27) it can be seen the comparison of the drug release of the CV coatings in PBS and in Methanol respectively.

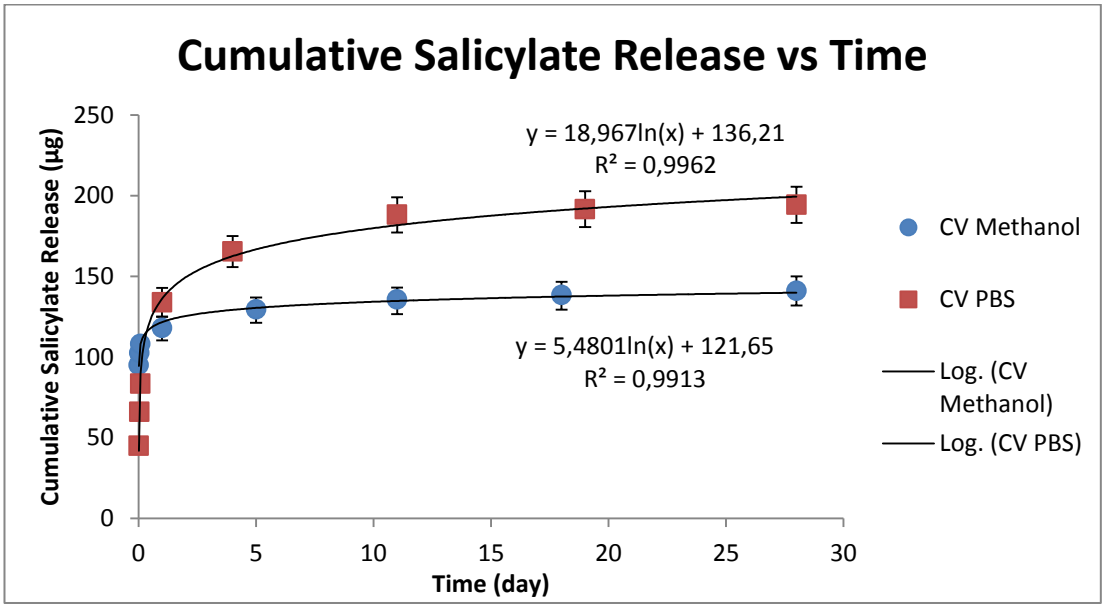


Figure 3.27: Comparison of Cumulative Salicylate release in Methanol/PBS versus Time profile of the 10 wires coated through CV method in 0.1M NaSa 0.1M (fresh py) solution.

From the figure (3.27) it is clear how the two coatings have different drug release trend lines. It can be seen that the CV coating had a higher first burst in methanol, which reached 120µg in 100 minutes time point, compared to the 90µg of drug release in PBS. After 5 days time point, both curves are seen to have a linear behaviour, in the range of 120-140µg in methanol and 160-180µg in PBS. Drug release percentage of the two coatings is shown in figure 3.28. It can be seen that the drug release percentage trendlines are very similar.

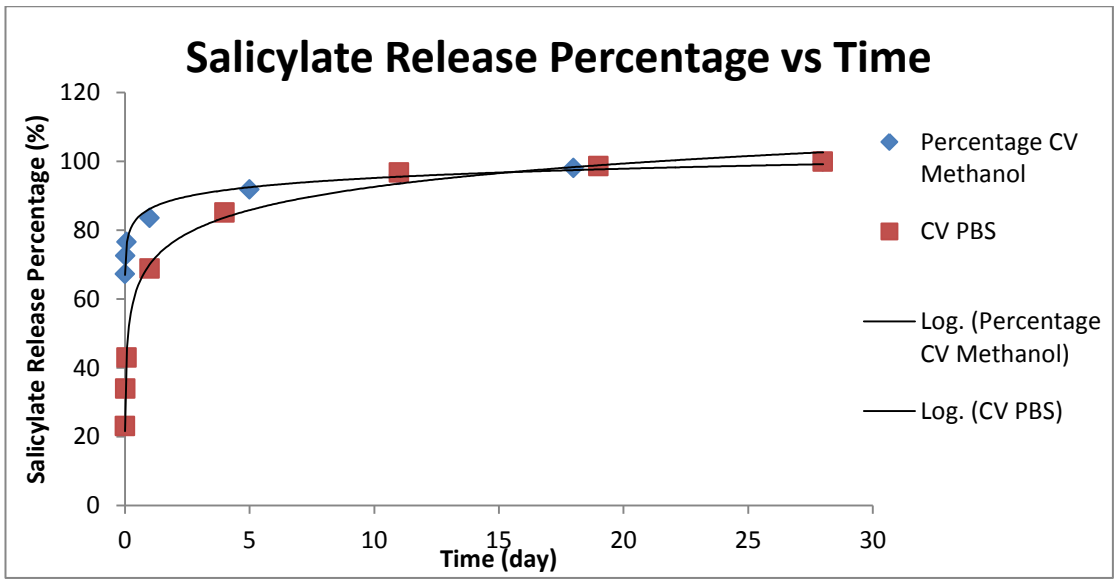


Figure 3.28: Comparison of Cumulative Salicylate release in Methanol/PBS percentage versus Time profile of the 10 wires coated through CV method in 0.1M NaSa 0.1M (fresh py) solution.

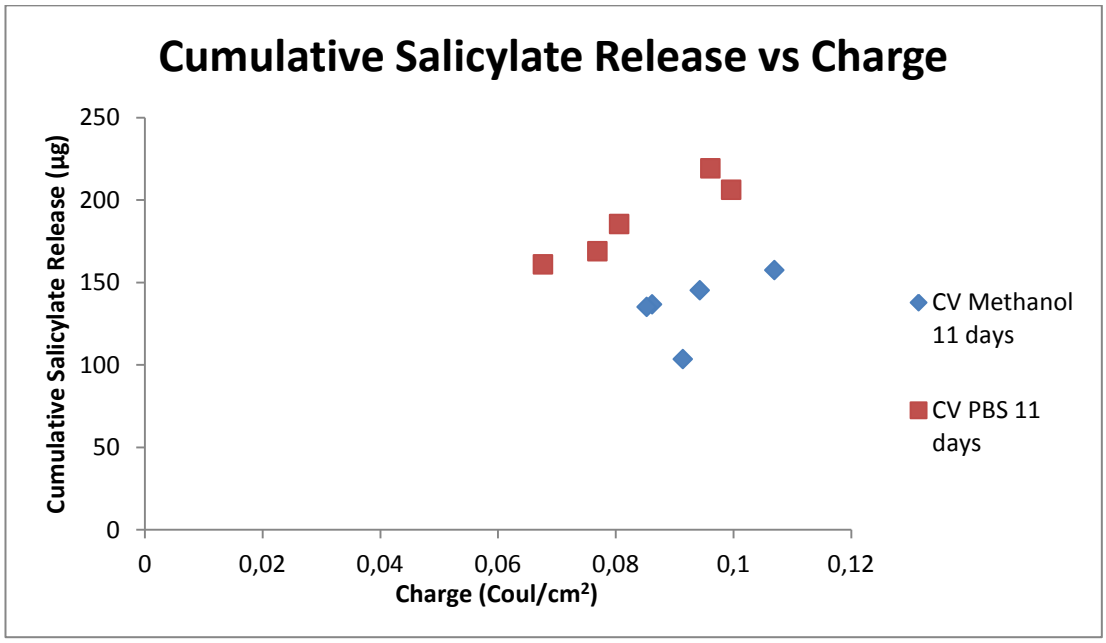


Figure 3.29: Comparison of Cumulative Salicylate release in Methanol/PBS versus Charge profile of the 10 wires coated through CV method in 0.1M NaSa 0.1M (fresh py) solution at 11 days time point.

The overall charge is very similar for both coatings. It may therefore be inferred that the differences of the two drug releases are due to the different solutions used (PBS vs Methanol).

CHAPTER 4. DISCUSSION

4.1 Introduction

In chapter 1 it was discussed how drug-eluting stents have improved the outcomes of stent implantation, introducing the ability to release an anti-proliferative drug into the vessel in order to inhibit the process of in-stent restenosis. Polymer coatings have been used to incorporate the drug and to modulate the drug release kinetics (Venkatraman & Boey, 2007). Unfortunately, this kind of stent has several disadvantages. They are found to lead to late stent thrombosis and delayed healing (Garg et al, 2010). The ideal drug-eluting stent must have good biocompatibility, optimal drug release profile and must be suitable to be implanted for a long term period.

Several studies have recently aimed to improve drug eluting stents design in order to improve clinical outcomes (Garg & Serruys, 2010), but very few of them (Arbizzani et al, 2007; Okner et al, 2007) have studied the potential use of the conducting polymer polypyrrole as a stent coating. Conducting polymers have some very interesting properties, such as good stability, conductivity, ease of synthesis and they can incorporate and then release particles (Guimard et al, 2007). Given these characteristics, they represent promising candidates as polymer coatings for use in a next generation stent. Therefore, this project set out to investigate the possibility of creating a conducting polymer stent coating, which has the ability to release drug over a therapeutically relevant period of time. In particular, the biocompatible conducting polymer polypyrrole was used to coat stainless steel wires, in order to mimic the stainless steel stents strut. To accomplish this overall objective, the specific aims of this project were:

- To produce a series of conductive polypyrrole coatings using different methods of electropolymerisation.
- To investigate the characteristics of the surface and the drug release profiles of the different coatings.

We wanted to examine if drug release profiles could be optimised by modulating the electropolymerisation methods used (Cyclic Voltammetry and potentiostatic) and to determine what effect any differences in surface characteristics may may have on the final drug release profiles achieved using each method.

4.2 Research Context

In this project we used the conducting polymer, polypyrrole to coat stainless steel wires, which were selected in order to mimic the stent strut material commonly used in existing drug-eluting stents. The two methods of electropolymerisation, potentiostatic and cyclic voltammetry, used to coat the wires were found to successfully produce a thin coating of polypyrrole onto the metal wires. The methodology used in this work was based on a previous study conducted by Arbizzani et al, 2007, but several improvements were introduced in the current investigation. In common with Arbizzani et al, in this project pyrrole was selected as the monomer in the solution used to coat the wire and salicylate was used as the doping substance within this solution. Stainless steel wires were used to mimic the standard stents. This is in contrast to Arbizzani et al, who used platinum discs and foils, materials which are not commonly found within drug-eluting stents. In the study conducted by Arbizzani et al, only the Potentiostatic method of electropolymerisation was used, applying a constant voltage of 0.8V and 9V. In this project, a similar voltage was applied for the potentiostatic method (0.9V) in order to demonstrate if we could achieve similar results to Arbizzani et al whilst using a more clinically relevant experimental model. It is also known that cyclic voltammetry electropolymerisation has been used to produce polypyrrole coatings (Okner et al, 2007; Shi & Zhitomirsky, 2010) and in this study this method was also used to produce a second set of coatings. The comparisons between the drug release profile achieved in each case represents a further novel aspect of the present study. In addition, in the limited number of studies in this field published to date, no comparisons have been made between the surface characteristics achieved between cyclic voltammetry and potentiostatic methods. In this project, the characteristics of the coating surfaces were investigated using SEM. This work is therefore one of the first to investigate differences in surface characteristics and to specifically consider

how they might impact final drug-eluting stent performance. Finally, this study has examined the effect of release medium on rate of drug release, and in a further advance the drug release was measured at 37°C to simulate the body environment, which contrasts with Arbizzani's work, where the experiments were performed at room temperature.

4.3 Polypyrrole Coatings Production

It was found that polypyrrole coatings can be successfully produced on the surface of stainless steel wires using potentiostatic electropolymerisation. The voltage level of 0.9V applied to achieve these coatings was consistent with the work of Arbizzani et al, 2007, and demonstrates that their approach can successfully be applied to stainless steel 316L. This is an important finding as this grade of stainless steel is very commonly used in medical implants, including coronary stents. Given that Okner et al, 2007 had previously demonstrated the potential of cyclic voltammetry as a polypyrrole coating method for stent applications, we went on to investigate if this method could be used in our model system. We found that polypyrrole coatings were achieved by cycling the applied voltage between 0.5V and 2V, which compares to a voltage range of -0.4V to 1.4V previously used by Okner et al to coat stainless steel plates.

4.4 Polypyrrole Coating Surface Characteristics

The coatings obtained through the different electropolymerisation methods showed differences in terms of surface properties. The coating obtained by NaCl solution showed a thick, rough dark surface, characterised by 'bumps' as showed in figure 3.8(a). The coatings produced through potentiostatic electropolymerisation using 0.1M NaSa 0.1M Py solution showed a smoother and lighter surface compared to the potentiostatic NaCl coating. The surface of the wires coated with existing pyrrole solution showed a lighter coating with some spots where the coating is not present (figure 3.9(a)).

The cyclic voltammetry coatings were produced by applying a voltage range of 0.5 to 2V for 5 cycles. The surface of those coatings appeared to be darker and thicker

than the potentiostatic ones. The surface presents bumps with an oval shape, as shown in the figure 3.10(a). The CV coatings produced with fresh pyrrole solution showed a lighter and more homogeneous surface, compared to the wires coated with existing Py solution.

No previous studies have made a comparison of the potentiostatic and cyclic voltammetry methods of coatings in terms of differences in surface characteristics. Referring to the study conducted by Shi & Zhitomirsky, 2010, the surface characteristics of the coatings were investigated using the SEM. Shi & Zhitomirsky used stainless steel wires and plates which were coated with pyrrole and either sodium salicylate or tiron (Shi & Zhitomirsky, 2010). The electropolymerisation method used in this paper to coat the electrodes was cyclic voltammetry within a potential range of -0.5 to +0.4V (Shi & Zhitomirsky, 2010), which differs to the potential used in this project (0.5 to 2V). The aim of that study was to understand the relationship between surface characteristics and the capacitive behaviour and the specific capacitance of these coatings, so not the potential biomedical application of the coatings (Shi & Zhitomirsky, 2010). In the study of Shi & Zhitomirsky, the SEM images of the coating surfaces of the wires showed similarities to the SEM images produced in this project. In both studies, the surface of the CV coatings showed a significant surface roughness, characterized by large particles (figure 3.12(a)). The SEM images of the cross-section area of the wires generated in both studies were helpful in better understanding the thickness of the different coatings produced.

The results obtained from the SEM analysis in the present study are interesting because they demonstrate that the method of the electropolymerisation influences the surface properties of the coating. They also indicate that the dopant ion used in the coating solution can have a significant impact on the surface characteristics achieved, with the smaller dopant ion (chloride) appearing to produce rougher surfaces than the larger salicylate dopant ion. Finally, the differences in the coatings produced when different stock solutions of pyrrole were used indicates the sensitivity of this coating approach to very small changes in the monomer solution.

4.5 Drug Release Profiles from Polypyrrole Coatings

Drug release profiles of the two different coatings, measured in PBS solution, were observed to be quite similar, in terms of percentage of cumulative drug release (figure 3.24). The main difference in drug release profile was seen to be related to the 'freshness' of the pyrrole solution used to produce the coatings. In fact, according to the graph shown in figure 3.24, the potentiostatic coating produced with existing pyrrole released less drug over a period of 28 days than the 'fresh' pyrrole coatings. This result was interesting and expected because the surface of this coating was seen to be not homogeneous and it was the only one that presented places where the coating was missing. On the contrary, the potentiostatic coating produced with fresh pyrrole solution showed a better drug release profile. The cyclic voltammetry coatings released a higher amount of drug over a period of 28 days compared to potentiostatic coatings. The CV coatings showed thicker and rougher surfaces, so these results suggest that there may be a relationship between the drug release profiles and the coating surface.

We measured drug release profiles also in methanol, in order to examine the effect of release medium on drug release. Different drug release profiles were obtained from the coatings placed in methanol solution. In fact, it was seen that the potentiostatic coatings did not release any drug over a period of 28 days. On the contrary, the CV coatings placed in methanol presented a percentage of drug release profile very similar to the CV coating placed in PBS (see figure 3.28). These results were interesting and unexpected. In fact, the current densities of the Pot wires placed in Methanol were similar to the wires placed in PBS (figure 3.29). It would suggest that the salicylate level in the coating should be similar. This finding demonstrates the importance of selecting an appropriate release medium to measure release. These results must be taken into account for further studies.

4.6 Study Hypothesis

In this project we wanted to investigate if drug release profiles could be optimised by modulating the electropolymerisation methods used and to determine if changes in surface characteristics may be related to changes in drug release profile. With the

experiments performed, the preliminary data generated indicate that the hypothesis of the study may be correct. In fact, we found tentative evidence to suggest that there is a relationship between the coating method, the characteristics of the surface and the eventual drug release profile achieved. As mentioned in the first chapter, the ideal drug-eluting stent must have good biocompatibility, optimal drug release profile and must be suitable to be implanted for a long term period. These results are important for the development of a new drug-eluting stent, because it is possible to use these findings in order to inform the development of an optimal drug release profile.

4.7 Study Limitations and Future Work

Although the results produced were reliable, as indicated by the low variability in the data produced and its similarity to comparable studies (Arbizzani et al, 2007), this project did however present some limitations. In the present study, the stainless steel wires were immersed in static solutions (PBS/Methanol), in order to measure the drug release profiles. Since stent coatings are exposed to flowing blood flow in the body environment, and future drug release experiments should be therefore performed with flow introduced into the system via a perfusion circuit.

As the amount of salicylate that was loaded onto each wire is unknown, this could be addressed by weighing the coatings and determining dopant ion uptake using the same methodology used by Arbizzani et al. in their study (Arbizzani et al, 2007).

The results obtained from the SEM imaging and from the drug release study suggest that there may be a relationship between the drug release profiles and the coating surface. As an example, the CV coatings released a higher amount of drug over a period of 28 days compared to potentiostatic coatings and at the same time they showed thicker and rougher surfaces. From these results we can assume that thicker coatings contain more drug in their matrix, and hence drug release is higher at 28 days. In terms of the roughness, perhaps it may indicate greater porosity which would provide access for the drug to be released out from the polymer matrix. Porosity and surface area were not measured in our experiments, so these measurements would likely be required to fully establish if the link between the

surface and drug release is genuine and if it is anything more than simply a relationship between thickness and drug release.

In addition, stainless steel wires were used in this work to mimic stent struts, but a wire is not ultimately a stent. During stent placement the stent is expanded, therefore it should be necessary to investigate further the behaviour of the coating during this process. Future studies are therefore required which would apply the coating technology directly to a bare metal stent to determine if the promising findings demonstrated here can be replicated to produce a drug-eluting stent.

Methanol solution, which was used as buffer solution to measure the drug release, was stored at room temperature. To allow more accurate comparison to the PBS release data, the methanol release could be performed at 37°C, to simulate body temperature.

4.8 Conclusions and Future Research Directions

In this project we found evidence to tentatively suggest that there may be a relationship between the coating method chosen, the characteristics of the surface and the drug release profile generated. By varying the parameters of the coating process, such as the coating method, voltage applied and coating solution, it should be possible in future to modify the drug release profile of the coating. The novel approach used in this work was to investigate the conducting polymer coating properties introducing improvements to the past studies. In fact, in the experiments, we tried to mimic the drug release from the stent in the body environment. The results achieved so far in this work might therefore have an important impact on a novel drug-eluting stent development.

In section 1.7, evidence that oxidative stress is produced at the site of stent implantation was presented. In particular, reactive oxygen species (ROS) may play an important role in the in development of in-stent restenosis (Azevedo et al, 2000). Since conducting polymers have been seen to act as antioxidants (Gizdavic-Nikolaidis, 2004), this could have important implications for the use of these polymers as stent coatings, given the potentially negative effect of oxidative stress on

outcomes following stenting. The DPPH assay has been used previously (Gizdavic-Nikolaidis, 2004) to test the ability of polypyrrole to act as a ROS scavenger. This method is commonly performed in methanol solutions, so the study of drug release in methanol that was conducted in this project was a preliminary study to determine if the surfaces produced had anti-oxidant capacity and to consider if methanol represented a suitable environment for measuring drug release. However, it is not clear if the anti-oxidant properties observed in these previous studies (Gizdavic-Nikolaidis, 2004) are retained when these polymers are coated onto metal substrates. This would therefore be the first future research avenue I would investigate.

BIBLIOGRAPHY

Abbott Laboratories. (2011). The XIENCEV® and XIENCEnano™ Everolimus Eluting Coronary Stent System Instructions for Use. Available on http://www.abbottvascular.com (accessed on 07 August 2012).

Acharya G., K. Park. (2006). Mechanisms of controlled drug release from drug-eluting stents. Advanced Drug Delivery Reviews 58:387–401.

Arbizzani Catia, Mastragostino Marina, Nevi Laura, Rambelli Laura. (2007). Polypyrrole: A drug-eluting membrane for coronary stents. Electrochimica Acta 52 3274–3279.

Bourke S. (2011). Conductive Polymers: Their use and possible application in coating drug-eluting stents, Master of Research Thesis, University of Strathclyde.

Bousalem S., A. Yassar, T. Basinska, B. Miksa, S. Slomkowski, A. Azioune and M. M. Chehimi. (2003). Synthesis, Characterization and Biomedical Applications of Functionalized Polypyrrole-Coated Polystyrene Latex Particles. Polym. Adv. Technol. 14, 820–825.

Boyle A., E. Genies, M. Fouletier. (1990). Electrochemical behavior of polypyrrole films doped with ATP aniuons. J. Elòectroanal. Chem. 279 179-186.

Dall'Olio A., G. Dascola, V. Varacco, V. Bocchi. (1968). Electron paramagnetic resonance and conductivity of an electrolytic oxypyrrole [(pyrrole polymer)] black. C R Acad Sci Ser C 267:433–5.

Forrester James S., Fishbein Michael, Helfant Richard, Fagin James, (1991). Journal of the American College of Cardiology 17:758-769.

Garg S., P. W. Serruys. (2010). Coronary Stent.Current Status. Journal of the American College of Cardiology Vol. 56, No. 10 Suppl S.

George P.M., A.W. Lyckman, D.A. LaVan, A. Hegde, Y. Leung, R. Avasare, C. testa, P. M. Alexander, R. Langer, M. Sur. (2005). Fabrication and biocompatibility of polypyrrole implants suitable for neural prosthetics. Biomaterials 26:3511-3519.

George Paul M, Lyckman Alvin W., LaVan David A., Hegde Anita, Leung Yuika, Avasare Rupali, Testa Chris, Alexander Phillip M., Langer Robert, Sur Mriganka. (2005). Fabrication and biocompatibility of polypyrrole implants suitable for neural prosthetics. Biomaterials 26:3511–3519.

Gizdavic-Nikolaidis M., Travas-Sejdic J., Bowmaker G.A., Cooney R.P. and Kilmartin P.A. (2004). Conducting polymers as free radical scavengers. Synthetic Metals. 140(2-3):225-232.

Goetz RH, Rohman M, Haller JD, Dee R, Rosenak SS. (1961). Internal mammary-coronary artery anastomosis. A nonsuture method employing tantalum rings. Journal of Thoracic Cardiovascular Surgery. Mar; 41:378–386.

Grüntzig AR, Senning Å, Siegenthaler WE. Nonoperative dilatation of coronary artery stenosis: percutaneous transluminal coronary angioplasty New English Journal of Medicine 1979;301:61.

Guimard N.K., N. Gomez, C. E. Schmidt. (2007). Conducting polymers in biomedical engineering. Progress in Polymer Science 32:876–921.

Gurunathan K., A. Vadivel Murugan, R. Marimuthu, U.P. Mulik, D.P. Amalnerkar. (1999). Electrochemically synthesised conducting polymeric materials for applications towards technology in electronics, optoelectronics and energy storage devices. Materials Chemistry and Physics 61:173-191.

Hansson GK & Hermansson A. (2011). The immune system in atherosclerosis Review. Nature immunology. Volume 12 number 3.

Haude M., Erbel R., Issa H., Meyer J. (1993). Quantitative Analysis of Elastic Recoil After Balloon Angioplasty and After Intracoronary Implantation of Balloon-Expandable Palmaz-Scbatz Stents. Journal of the American College of Cardiology Vol. 21, No. 1. 26-34.

Hepel M., Mahdavi F. (1997). Application of the electrochemical quarz crystal microbalance for electrochemically controlled binding and release of chlorpromazine from conductive polymer matrix. Microchemistry Journal. 56:54-64.

Kaminnyi A.I., Lankin V.Z., Samko A.N., Sozykin A.L., Provatorov S.I.,. Konovalova G.G, Perepelitsa E. I., Tikhaze A. K., Polevaya T. Yu.,. Kukharchuk V. V, and Yu. N. Belenkov (2005). Low daily dose of antioxidant probucol decreases incidence and severity of restenosis after transluminal coronary balloon angioplasty. Bullettin of Experimental Biology and Medicine 139:183-5.

Konturri K., P. Pentti, G. Sundholm. (1998). Polypyrrole as a model membrane for drug delivery. Journal of Electroanalitical Chemistry 453:231-238.

Lefer D.J., D.N. Granger. (2000). Oxidative stress and cardiac disease The American Journal of Medicine, Volume 109, Number 4, pp. 315-323(9).

Lowe H. C., S. N. Oesterle, L. M. Khachigian. (2002). Coronary In-Stent Restenosis: Current Status and Future Strategies. Journal of the American College of Cardiology Vol. 39, No. 2.

Ludman Peter F. BCIS Audit Returns Adult Interventional Procedures. Jan 2010 to Dec 2010.

Maggi G.C. (1988) Ischemia miocardica e nuove acquisizioni. Casa editrice CHIESI, Library in Cardiology p.46.

McFadden E.P., E. Stabile, E. Regar, et al., (2004). Late thrombosis in drug-eluting coronary stents after discontinuation of antiplatelet therapy, Lancet 364:1519–1521.

Miller L.L. & Q. X. Zhou. (1987). Poly(N-methylpyrrolylium), poly(styrenesulfonate). A conductive, electrically switchable cation exchanger that cathodically binds, and anodically releases dopamine. Macromolecules 20:1594-1597.

Misra P., P. C. Reddy, Shukla D., Caldito G. C., Yerra L., Aw T. Y. (2008). In-Stent Stenosis: Potential Role of Increased Oxidative Stress and Glutathione-Linked Detoxification Mechanisms. Angiology. Volume 59 Number 4:469-474 August/September.

Morrison D.A., Berman M, El-Amin O, McLaughlin RT, Bates ER. (2007) Emergency percutaneous coronary intervention (PCI) for the care of patients with ST-elevation myocardial infarction (STEMI). Minerva Cardioangiology. Oct;55(5):593-623.

Nilsen D.W.T., Melberg T., Larsen A.I., Barvik S., Bonarjee V. (2006). Late complications following the deployment of drug eluting stents. International Journal of Cardiology, 109(3):398-401.

Okner Regina, Domb J. Abraham and Mandler Daniel. (2007). Electrochemical Formation and Characterization of Copolymers Based on N-Pyrrole Derivatives. Biomacromolecules, 8:2928-2935.

Petit Michel A., Plichon Vincent, Colin Claude. (2000). A CV:PBD coupled study of ionic exchanges at poly(pyrrole) films in phosphate buffered solutions. Electrochimica Acta 45 1953–1961.

Ramanath Vijay S., Brown Jeremiah R.,David Malenka J., DeVries,James T., Sidhu Mandeep S., Robb John F., Jayne John E., Hettleman Bruce D., Friedman Bruce J., Niles Nathaniel W., Kaplan Aaron V.,and Craig A. Thompson (2010).Outcomes of Diabetics Receiving Bare-Metal Stents Versus Drug-Eluting Stents. Catheterization and Cardiovascular Interventions 76:473–481.

Ramanaviciene Almira, Asta Kausaite, Stasys Tautkus, Arunas Ramanavicius. Biocompatibility of polypyrrole particles: an in-vivo study in mice. Journal of Pharmacy and Pharmacology Volume 59, Issue 2, pages 311–315, February 2007.

Raval A., J. Parikh and C. Engineer. Mechanism of controlled Release kinetics from medical devices. Brazilian Journal of Chemical Engineering. Vol. 27, No. 02, April - June, (2010) pp. 211 – 225.

Revell P.A., M. Braden, M.A.R. Freeman. Review of the biological response to a novel bone cement containing poly (ethyl methacrylate) and n-butyl methacrylate, Biomaterials 19 (1998) 1579–1586.

Saito Shigeru, Hosokawa George, Tanaka Shinji, and Nakamura Sunao, for the PASTA Trial Investigators. Primary Stent Implantation Is Superior to Balloon

Angioplasty in Acute Myocardial Infarction: Final Results of the Primary Angioplasty Versus Stent Implantation in Acute Myocardial Infarction (PASTA) Trial. Catheterization and Cardiovascular Interventions 48:262–268 (1999).

Schwartz R.S., Edelman E., Virmani R., Carter A., Granada J.F., Kaluza G.L., Chronos N.A., Robinson K.A., Waksman R., Weinberger J., Wilson G.J., Wilensky R.L. (2008). Drug-eluting stents in preclinical studies: updated consensus recommendations for preclinical evaluation. Circulation Cardiovasc Intervention. Oct;1(2):143-53.

Serruys Patrick W. and Gershlick Anthony H. Handbook of drug-eluting stents. Taylor & Francis, June (2005) - 336 pages.

Shi C., Zhitomirsky I. (2010) Electrodeposition and capacitive behavior of films for electrodes of electrochemical supercapacitors. Nanoscale Research Letters 5:518-523.

Simon D. Inflammation: The Key Element in the Biology of Restenosis. Inflammation Summit, TCT 2003. Inflammation Summit. TCT 2003.

Sousa J. E., Costa Marco A., Abizaid Alexandre *et al.* (2001). Lack of Neointimal Proliferation After Implantation of Sirolimus-Coated Stentsin Human Coronary Arteries: A Quantitative Coronary Angiography and Three-Dimensional Intravascular Ultrasound Study. Circulation, 103:192-195.

Steinberger Julia and Daniels Stephen R. (2003). Obesity, Insulin Resistance, Diabetes, and Cardiovascular Risk in Children: An American Heart Association Scientific Statement From the Atherosclerosis, Hypertension, and Obesity in the Young Committee (Council on Cardiovascular Disease in the Young) and the Diabetes Committee (Council on Nutrition, Physical Activity, and Metabolism). Circulation, 107:1448-1453.

Stone Gregg W. Rizvi Ali, Newman William *et al.* (2010). Everolimus-Eluting versus Paclitaxel-Eluting Stents in Coronary Artery Disease. New English Journal of Medicine 362:1663-1674.

Svirskis D. J. Travas-Sejdic, Rodgers A., Garg S. (2010) Electrochemically controlled drug delivery based on intrinsically conducting polymers. Journal of controlled Release 146 :6-15.

Venkatraman Subbu, Boey Freddy. (2007) Review. Release profiles in drug-eluting stents: Issues and uncertainties. Journal of Controlled Release 120, 149-160.

Vernitskaya T.V., Efimov O. N., (1997).Polypyrrole: a conducting polymer; its synthesis, properties and applications. Russian Chemical Reviews 66 (5) 443 - 457

Virmani R., Guagliumi G., Farb A., Musumeci G., Grieco N., Motta T., Mihalcsik L., Tespili M., Valsecchi O., Kolodgie F. D. (2004). Localized Hypersensitivity and Late Coronary Thrombosis Secondary to a Sirolimus-Eluting Stent: Should We Be Cautious? Circulation 109:701-705.

Watt J, Wadsworth R, Kennedy S, Oldroyd KG. (2008). Pro-healing drug-eluting stents: a role for antioxidants? Clinical Science (London) 114 (4) 265-73.

Welt Frederick G.P. & Campbell Rogers. (2002) Inflammation and Restenosis in the Stent Era. Arteriosclerosis, Thrombosis and Vascular Biology. 22:1769-1776.

WHO international. Cardiovascular diseases (CVDs), Fact sheet N°317. September 2011. Available on http://www.who.int (accessed on 01 August 2012).

Zinger B., Miller L.L. (1984) Timed release of chemicals from polypyrrole films. Journal American Chemistry Society 206:6861 – 6863.