Bioengineering Unit

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Optimising drug release from coronary stents: a role for conducting polymers?

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This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

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ABSTRACT

Coronary artery disease (CAD) is the leading cause of death worldwide. It is characterised by the narrowing of the coronary vessels by atherosclerotic plaque formation. The two main approaches to treat CAD are by bypass grafting and Percutaneous Coronary Intervention (PCI). Currently the most widely used approach for PCI involves the use of drug-eluting stents (DES). Despite their demonstrated efficacy, these DESs have still been prone to suffer from cases of restenosis, have a risk of thrombosis formation and are not suitable for all patient types. Their main weaknesses stem from polymer coatings that are believed to be pro-inflammatory and the elution of drugs which may inhibit the natural healing process. The use of Conducting Polymer coatings for stents have been suggested because of their biocompatible surfaces and ability to elute a range of drugs. The current study produced a series of polypyrrole conducting polymer coatings on stainless steel wires and bare metal stents using Electropolymerisation. The coating properties and 30 day drug release profiles were then assessed. The Pyrrole and Salicylate concentrations used in the electropolymerisation solution were varied to observe their effect on coating properties and drug release profile. The results showed that the electropolymerisation method selected could successfully produce coatings on the stainless steel wires and bare metal stents. These coatings were shown to be able to elute Salicylate drug over a 30 day period. The data demonstrated that both Pyrrole and Salicylate were needed for coating formation and that the ratio of these two components affected the coating properties. Some issues noted with the coatings included their fragility, large thicknesses and variable release profiles. In conclusion, it was found that conducting polymer coatings may be a viable option for producing drug-eluting stents but further investigation is necessary to determine the experimental conditions required to produce the coating with optimal surface and drug release properties.

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CHAPTER ONE

INTRODUCTION

1.1 Coronary Artery Disease

Coronary artery disease (CAD) is the leading cause of death worldwide. The prevalence of the disease has shown an increase over the last century. Coronary Artery Disease (CAD) health care accounts for £1.7 billion in cost to the NHS each year (Liu et al, 2002). Factors not included in these statistics, are the effects on the quality of life of patients - encountered through the symptoms attributed to CAD.

1.1.1 Mortality and Morbidity

Coronary artery disease presents in the form of angina, in which the patient suffers from chest pain on exertion or stress; this may include exercise, physical demand, or anxiety. These symptoms may pass with rest or may require emergency and long term medication such as Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) like Aspirin. The greatest risk resulting from this disease is the increased chance of suffering from a myocardial infarction, which, depending on the scale of the attack, can prove fatal (Thomas et al, 1988).

1.1.2 Atherosclerosis

Coronary artery disease occurs as a result of the coronary vessels, which supply blood to the heart, becoming stenosed (narrowed) by the build up of fatty deposits within their lumen. This narrowing reduces the capacity for haemodynamic blood flow and hence impairs oxygen supply to the heart muscle. The lack of nutrient supply accounts for the angina like symptoms and the risk of myocardial infarction. The build up of fatty deposits is described as the development of atherosclerosis and is known to have a well defined pathological pathway. Atherosclerosis develops over many years and is often described as a multifactorial disease. Genetic profiling has highlighted a genetic predisposition in certain groups of individuals to developing atherosclerosis, however, lifestyle issues are often viewed as the substantial factor in accelerating and promoting the development of the disease. These issues often involve a sedentary lifestyle alongside a diet rich in saturated fats and sugars (Bonow et al, 2011). Alongside these issues, the likelihood of developing CAD is significantly increased in people with underlying medical conditions, such as diabetes, hypertension and hypercholesterolaemia (Culling et al, 2007).

1.1.3 Primary, Secondary, Tertiary Prevention

Treatment for CAD is necessary to alleviate the angina type symptoms and to reduce the risk of myocardial infarction. The treatment approach for coronary artery disease usually occurs in three main stages. Primary Prevention focuses on preventing or reducing the scale of atherosclerosis that is present. This fundamental approach involves reducing general atherosclerosis development risk and hence, eliminating any symptoms of CAD and the increased risk of myocardial infarction. This approach focuses on changes which are possible in a person's environment and lifestyle. Secondary prevention focuses on targeting and reversing the development of the atherosclerotic plaque within the blood vessels. This may be achieved by following similar strategies to those presented in primary prevention and furthered by the direct treatment of hypercholesterolemia and treatment of other co morbidities such as hypertension and diabetes. However, one of the limitations of coronary artery disease therapy is that once the fatty deposition has begun in the vessels, it is often difficult to reverse or remove this build up. Certain strategies have been tested to remove the atheroma from the diseased vessel but with no real success (Topol et al, 1993). Any hopes of reversing atherosclerosis have until now not been realised. Hence, the main arm of treatment for coronary artery disease is by correcting the problem caused by atherosclerosis, namely the narrowing of the vessels. This approach aims to restore the supply of nutrients to the heart muscle by performing a revascularisation procedure. Coronary Artery Bypass Grafting (CABG) was the first revascularisation procedure to become widespread and it remained the gold-standard treatment for much of the 20th Century.

1.2 Coronary Artery Bypass Grafting

Coronary artery bypass grafting (CABG) sets out to redirect the blood flow from the stenosed vessels to the intended cardiac muscle via supplementary vessels; which are surgically attached from the pre stenosed end of the vessel to a path further down the vessel – in an attempt to bypass the blockage. CABG has historically been the treatment method of choice, because of its high success in revascularising the diseased tissue (Morton et al, 2007). The technique and approach has undergone substantial development since its introduction, with improved surgical techniques, the use of more compatible supplementary vessels, improved anaesthesia, advanced methods of arresting the heart, development of imaging modalities providing greater precision, and the provision of more specific anticoagulation therapies. Through such developments, CABG became the gold standard treatment for CAD and remains so to this day for certain patient groups and lesion types (Serruys et al, 2009). The main benefit that this approach offers is an increased rate of successful revascularisation in difficult patients. These include patients with multivessel disease, bifurcation lesions, torturous lesions, and small vessel lesions. The method of CABG ensures that these lesions may be bypassed

successfully. However, CABG has historically been a difficult procedure for patients to undergo due to the nature of the invasive surgery. The potential difficulties arise from the procedure of open heart surgery with possible cardiac arrest. Generally the potential complications with undergoing the surgery and problems of suffering with bleeding is present in all patients, however, this is exacerbated in certain groups of patients: namely the elderly population (70 years and above) and those suffering with comorbidites, including those at risk of thrombosis, which often cover the patient groups most in need of the treatment (Stutz, 2009).

These risks and contraindications for certain patient groups, along with the successful emergence of interventional cardiology has led to the development of possible therapy routes with percutaneous coronary intervention (PCI). Historically, PCI was viewed as a technique for patients unsuitable for CABG (elderly, fragile, comorbidities), as a means of supplying symptomatic relief (Micheals & Chatterjee, 2002). However, such has been its development that the technique is beginning to rival the elective choice of CABG in major lesion conditions, not only for symptomatic relief but as a first choice treatment (Motovska et al, 2010).

1.3 Percutaneous Coronary Intervention

Percutaneous coronary intervention in its infancy was viewed as a backup option for patients suffering with coronary artery disease. If the clinician decided that the patient was not suitable for CABG, PCI was viewed as a viable conservative option. The less invasive nature of the procedure made it suitable for patients who otherwise could not undergo surgery. However, it was only suitable for patients suffering from simple single lesions. Balloon coronary angioplasty as a percutaneous procedure was pioneered in 1977 as a means of reversing the narrowing within the target vessel by physical expansion via balloon inflation (Grüntzig et al, 1979). The balloon angioplasty procedure involves passing a wire through an auxillary artery, often the femoral or radial, to the target site of the lesion. This percutaneous route dramatically reduces the size of surgical approach relative to that of CABG. Stent expansion is achieved through the balloon inflation via an external pump. The level of expansion is dependent on arterial size and lesion type. The lesions targeted were those in single vessels, with large enough diameters to accept the passage of a balloon. The procedure involved inflating the balloon over the length of the lesion, in an attempt to compress the atheroma against the intimal wall of the vessel and also expand the general diameter of the vessel, in the hope of increasing the luminal diameter available for haemodynamic blood flow. Positive outcomes were demonstrated in select patients, with successful revascularisation of the diseased vessel being achieved and a consequent reduction in symptoms. Problems arose in these patient groups however, with major concern being the risk of acute vessel closure. Due to the nature of the procedure, the vessels were susceptible to elastic recoil at any time – which meant that the

patient was still susceptible to suffering from an unexpected myocardial infarction with similar if not greater risk than before (Waller, 1989). In addition to this, a risk of restenosis developing was discovered. In this case, smooth muscle cells and extracellular matrix would build up inside the intimal layer and narrow the vessel. This restenosis was noted as being more accelerated than the natural course of atherosclerosis and would often build up over a 6 month period post procedure (Holmes et al, 1984). Studies demonstrated that patients undergoing coronary balloon angioplasty on a diseased vessel would suffer from restenosis in 30-50% of cases (Gruentzig et al, 1987; Hirshfeld et al, 1991). This risk was attributed to a variety of effects including, elastic recoil within the vessel, platelet mediated thrombus formation, proliferation of smooth muscle cells and vascular remodelling (Waller, 1989). Patients suffering from restenosis would often require a repeat revascularisation treatment, resulting in either a further PCI procedure or if necessary CABG. Despite balloon angioplasty's success in revascularising certain patient groups, problems still remained which made such a procedure only viable for a limited number of patients and it was at best a conservative approach for patients that were too high risk for surgery.

1.4 Bare Metal Stents

It was proposed that the issue of acute vessel closure due to elastic recoil could be prevented by the use of a metal scaffold, namely a stent. Initially, metal stents were developed and tested on animal models with reasonable levels of success (Palmaz et al, 1985; Schatz et al, 1987). The use of stents in PCI became a reality following the results published by the BENESTENT and STRESS trials (Serruys et al 1994; Fischman et al, 1994). The BENESTENT trial consisted of 520 patients being entered in a multicentre randomised study comparing the safety and efficacy of metal stents versus balloon angioplasty, for patients suffering from a single new lesion in a coronary artery which produced stable angina symptoms (Serruys et al, 1994). The trial used a 6 month follow up period and measured primary clinical end points of death, myocardial infarction and revascularisation, whilst primary angiographic end points focussed on luminal diameter post procedure and rate of restenosis (defined as greater than 50% stenosis of luminal diameter) at follow up. End points of death and myocardial infarction returned with similar rates in each patient group, however, the rates of revascularisation and restenosis were significantly less in the metal stent group. These findings were attributed to the ability of the metallic stent to increase the luminal diameter of the vessel to a greater extent than the balloon could achieve (de Jaegere et al, 1993; Rensing et al, 1990). At around the same period as the BENESTENT trial, the STRESS trial was published, which again used data from a multicentre prospective randomised study to compare the use of metal stents against balloon angioplasty in treating patients with a symptomatic new lesion within their coronary circulation (Fischman et al, 1994). It was suggested, based on previous smaller scale studies, that the stents may

act to reduce the potential for restenosis by decreasing elastic recoil within the vessel and sealing intimal flaps by providing a metallic scaffold (Ellis et al, 1992; Savage et al, 1992). 410 patients were recruited and randomised to either treatment arm, with a 6 month follow up and end point focus on rates of death, myocardial infarction, revascularisation, restenosis and the measured luminal diameter. The clinical events of death and myocardial infarction were not significantly different in their rates, however, the number of revascularisations and restenosis rates were significantly less in the stent group, with a consistently larger luminal diameter increase and maintenance of this gain over the follow up period. Again, the successful results of reduced stenosis were attributed to the significantly larger luminal diameter achieved by stenting. The results from the BENESTENT and STRESS trials were significant in supporting stent use over PCI by sole balloon angioplasty procedure. The stent was believed to scaffold the artery, stabilise the plaque and help to prevent arterial recoil within the target vessel (Morton et al, 2007). As a result, the opportunity for abrupt vessel closure was eliminated and the occurrence of restenosis in the target vessel reduced, both contributing to the reduced target vessel revascularisation rates. However, further studies still demonstrated that restenosis would occur within the stent structure, albeit at a reduced rate.

1.4.1 In-stent restenosis

The major area of concern with the introduced bare metal stents was the formation of in-stent restonosis (ISR). ISR is when the luminal diameter of the stented vessel becomes stenosed and narrowed once more. While similarities exist between the different diseases, the pathophysiology of ISR is considered to be distinct from the process of stenosis and atherosclerosis, with neointima formation being the major factor in the development of ISR. ISR follows a pattern of neointimal hyperplasia and vascular remodelling, with ISR occurring in 15-60% of patients receiving a bare metal stent (Serruys et al, 1994; Fischman et al, 1994).

1.4.1.1 Mechanisms of In-stent restenosis

As a result of the relatively high rates of ISR, significant research effort has been dedicated to gaining a greater understanding of the cellular mechanisms responsible for this response to stenting. A study in 1999 on the pathology and histology of restenosis within stents, aimed to describe this process (Farb et al, 1999). It was proposed that there was a time course over which the vessel and accompanying cells respond to the implantation of a bare metal stent. This was demonstrated by analysing autopsy samples of patients who had been recently implanted with stents. Data were collected at varying time points, from the first few days following implantation to time points beyond 30 days. From this and similar studies, a time line of the key biological responses to stent insertion

has been constructed. In the early stage, 3 days or less, platelet deposition is observed to take place over the stent struts. Acute inflammatory cells (neutrophils) are recruited very early as a part of an inflammatory response to the metallic stent. The chronic inflammatory cells (lymphocytes, macrophages) are noted as being present throughout the duration of the restenosis formation. A neointima begins to form after these early periods, usually after 11 days, were smooth muscle cells are recruited and begin to form layers over the stent struts. After a sufficient period (up to 30 days) the neointima becomes encased in a proteoglycan matrix which stabilises its formation. The picture early after stent implantation (first 10 days) is the association and presence of fibrin, platelets and acute inflammatory cells at the stent strut sites. The level of inflammatory response was shown to be related to the nature of the stent-arterial wall interface and degree of injury, with a greater inflammatory response being observed in patients with implanted stents sitting within the lipid core of the plaque or damaging the media of the vessel (Carter et al, 1996). The pathological findings in the autopsy study by Farb et al and previous porcine models (Carter et al, 1994) have supported this observation. Further studies testing stenting in experimental porcine models have demonstrated a similar picture (Schwartz et al, 1992). In these models, 24 hours after implantation, thrombi begin to form over the stent struts and have been shown to be composed of fibrin, trapped erythrocytes and acute inflammatory cells (Carter et al, 1994). After 7 days the thrombus becomes more organised with neutrophils and macrophages present. From 14 to 28 days smooth muscle cells become the predominant cell type with few chronic inflammatory cells remaining. A similar study of the pathological findings of the tissue response to coronary stenting aimed to characterise the histological and immunohistochemical interactions to elucidate the mechanism for restenosis formation (Grewe et al, 2000). The study examined 31 human specimens post mortem and studied samples obtained from an implantation period of 25 hrs up to 340 days. Based on these histological samples a picture was developed of the stages in the neointima growth in relation to time. 12 of the 31 stents were incorporated with a neointima. The investigators aimed to establish whether the phased basis for neointimal growth was a correct model. They detail a 2 phase development path for neointimal generation, namely the Phase of Reparation and the Phase of Proliferation. The initial phase of reparation describes the formation of an intramural haematoma as a response to an initial rupture of the plaque or dissection of the vessel's media. This early thrombus is loose without any clear structure, with a composition of red blood cells, neutrophils and thrombocyte aggregates – this is described as the early neointima. The 2nd phase, that of proliferation, involves a reduction of inflammatory infiltrates over the first 28 days, and an increase in smooth muscle cells and extracellular matrix making up the composition of the neointima. The neointima will undergo progressive solidification of its structure and its volume will increase through this process. If the neointimal growth proceeds along this pathway, then eventually the treated vessel would be come stenosed again, fulfilling the picture of in-stent restenosis (Komatsu et al, 1998). The findings within the study were able to demonstrate a similar occurrence with two defined phases. As

aforementioned, the initial phase involves coverage of the implantation zone by a membranous thrombus, this is followed by infiltration of smooth muscle cells which secrete the extracellular matrix. Following this middle aspect, the 2nd phase involves the increase in number of smooth muscle cells and matrix, which increases the overall volume of the neointima. It is suggested that the initial inflammatory response; which initiates the infiltration of cellular components, is a response to trauma to the lipid plaque or coronary vessel. Following this, the inflammatory response and neointimal growth is perpetuated by the presence of the stainless steel metal and continued infiltration of 'inflammatory' cells (Hanke et al, 1995). Given the clear mechanistic development of in-stent restenosis, treatment strategies have involved targeting various aspects of that process.

1.4.1.2 In-stent restenosis treatment strategies

Initial attempts to reduce the inflammatory response therapeutically involved the administration of pharmacological agents. The agents that were trialled often reflected either a general inflammatory target or specific targets against cells highlighted in the chain of cellular responses. Some evidence suggested that if the mural thrombus formation may be controlled then the possibility of neointimal hyperplasia would be decreased (Bapabulle & Eisenberg, 2002). Studies and trials have demonstrated however, no significant impact on post percutaneous coronary intervention restenosis rates. The use of anitplatelet agents, such as Clopidogrel, has not reliably resulted in the reduction of restenosis rates in clinical trials (Kastrati et al, 1997). Similarly, the systemic administration of corticosteroids; aimed at controlling the inflammatory process, has not shown decreased restenosis rates (Lee et al, 1999). It is assumed that the limited effect of systematically administered agents may be due to the inadequate drug concentrations achieved at the site of stent insertion. As a result of their limited success in decreasing the rates of in-stent restenosis and the side effects that accompanied the use of systemic agents, their use was not established in clinical practice. Following on from these findings, the development of drug-eluting coatings for stents was proposed as a more efficient manner of targeting the processes of restenosis by eluting a drug of choice at the stent insertion site. The desire was to produce a coating that would not only make the stent more biocompatible in its setting (to reduce the inflammatory response) but also one which would release a drug at the site of action. This release at the site of action would ensure a high local concentration of agent in the diseased artery without a supposed risk of systemic toxicity.

1.5 Drug-eluting stents

Drug-eluting stents are stents which are coated with a synthetic polymer blended with a biologically active agent (commonly a drug). The polymer coating acts as a drug reservoir to allow the elution of

the active agent into the injured arterial tissue over a period of several weeks. Three main drug types were investigated as sources for the agent: anticoagulants, corticosteroids and antimitotic agents. A limited number of studies have examined the use of anticoagulant agents (heparin, hirudin) on drug-eluting stents and these have shown a positive effect on the subacute thrombus formation with variable reduction in neointimal hyperplasia (De Scheerder et al, 1997; Hardhammar et al, 1996; Ahn et al, 1999). A number of clinical trials of these drug-eluting stents have shown similar results, with the heparin coated stents being well tolerated and producing lower rates of subacute thrombosis when compared to uncoated stents. However, they did not have a significant impact on either major adverse cardiac events or restenosis rates (Babapulle & Eisenberg, 2002). Similarly, the results from animal studies investigating corticosteroid coated stents and their effect on neointimal hyperplasia have been unimpressive (Babapulle & Eisenberg, 2002). There are no published clinical trials evaluating the impact of corticosteroid coated stents. The most successful agent group used in drug-eluting stents have been the antimitotic agents, namely Siroliumus (Cypher), and its derivatives, Everolimus (Endeavour), Xience, Tacrolimus, Biolimus, and Paclitaxel (Taxus) releasing drug-eluting stents. Both animal studies and human clinical trials have shown positive significant results in terms of restenosis rates (and reduced revascularisation procedures) when compared to bare metal stents.

1.5.1 Sirolimus and Paclitaxel

The two drug-eluting stents that have been shown to be most effective in large randomised trials are the Sirolimus and Pacltaxel eluting stents. The CYPHER trial demonstrated that the Sirolimus (also known as Rapamycin) eluting reduced the observed rates of restenosis in patients implanted with this stent compared to that of bare metal stents (Morice et al, 2002). The drug itself is believed to act via its immunosuppressant and antimitotic properties. The drug binds to specific cytosolic proteins and inhibits the DNA synthesis of the cell. Its efficacy results from it inhibiting the protein mTOR (Mammalian Target of Rapamycin), which ultimately induces cell cycle arrest in late G1 phase and consequently arrests smooth muscle cell growth (McAlister et al, 2002).

The effectiveness of Paclitaxel on a polymer based stent coating was demonstrated in the large scale TAXUS trial, which again showed favourable results for restenosis and revascularisation rates when compared to the equivalent bare metal stent (Grube et al, 2003). Paclitaxel's primary mechanism of action is thought to be its microtubule stabilising effects, which shifts the targeted cells cytoskeleton equilibrium towards assembly, leading to reduced vascular cell proliferation, migration and signal transduction (Bharadwaj & Yu, 2004). In turn it prevents the expansion and formation of the neointimal layers.

A recent meta-analysis attempted to pool data comparing the drug-eluting stents of Sirolimus and Paclitaxel against the bare metal stent equivalent in patients elected for PCI as a result of a coronary lesion (Roiron et al, 2006). Data from 8987 patients were made available from 19 different randomised controlled trials performed over the period of 1996 to 2005. The primary end points of interest were rates of restenosis (defined as less than 50% of luminal diameter patent at 6 months follow up) and major adverse cardiac events (including death, myocardial infarction and revascularisation rates). Angiographic restenosis rates were shown to be significantly lower in the drug-eluting stent group when compared to that of the bare metal stent group (10.5% vs 31.7% with an Odds ratio of 0.25 [0.22 to 0.29, $p<0.001$] in favour of the drug-eluting stent group). The Meta analysis also demonstrated a significant heterogeneity within the drug-eluting stent group between Sirolimus and Paclitaxel, with Sirolimus presenting an odds ratio of 0.14 vs the bare metal stent group against Paclitaxel's 0.35. Implying, that despite both stent types achieving a reduced restenosis rate, this was more marked with the Sirolimus type. The events of major adverse cardiac event (MACE) were also significantly different, with 19.9% in the bare metal stent group and 10.1% in the drug-eluting stent group, with the difference being driven by the increased need for target lesion revascularisation for restenosis in the bare metal stent group. Again Sirolimus was shown to produce a larger reduction in MACE then the Paclitaxel group.

A further multi centre randomised controlled trial compared the impact of drug-eluting stents and bare metal stents on in stent late loss. In stent late loss (ISLL) is defined as the difference between the luminal diameter immediately after coronary stenting compared to that obtained at angiographic follow up (often after 6 months). This provides a more quantitative picture of restenosis development risk. 8645 patients were included in the pooled data, 4320 had undergone bare metal stent treatment and 4321 drug-eluting stent treatment. The study compared the difference in mean ISLL between the 2 groups. In all the trials, it was shown that the mean ISLL was significantly lower for patients allocated to the drug-eluting stent group. This difference highlighted the degree of inhibition of neointimal hyperplasia achieved by using the drug-eluting stents in place of bare metal stent therapy. Again, the trials showed a reduced level of target lesion revascularisation in the drug-eluting stent group than compared with the bare metal stent group. Also, Sirolimus was shown to be superior then Paclitaxel in the rates of ISLL and revascularisation reduction (Moreno, 2007).

Currently drug-eluting stents are widely used in the clinical practice of treating patients with coronary artery disease, with approximately 2.5 million drug-eluting stents implanted each year worldwide (Business Week, 2005). Several other randomised studies have shown that drug-eluting stents reduce the risk for restenosis from about 30% in bare metal stent to less than 10% (Babapulle et al, 2004).

1.5.2 Stent thrombosis

Despite the number of successful trials demonstrating the efficacy of drug-eluting stents use, safety concerns have also been highlighted. Thrombotic events remain the primary cause of death after percutaneous coronary intervention (Schomig et al, 1996). An observational cohort study set out to observe the rates of thrombosis following stent implantation and to establish the associated risk factors (Lakovou et al, 2005). 2229 patients were recruited (1062 on Sirolimus-eluting stent and 1167 on Paclitaxel-eluting stent) with a 9 month follow up. The occurrence of stent thrombosis was defined as angiographic documentation of partial or total stent occlusion within 30 days after implantation or the presentation of sudden cardiac death or postprocedural myocardial infarction as a result of a thrombotic event not related to another coronary lesion. The results showed that 1.3% of the patients on the procedure (29 patients) had suffered from stent thrombosis, 14 of these were subacute and the remaining 15 late-stage. Stent thrombosis is a major concern in these groups of patients, often they are generally compromised in terms of diseased vessels with risk factors of embolic development, and the implantation of a stent often implies greater risk of emboli formation, inflammation, restenosis and thrombotic development. Antiplatelet therapy remains the main strategy in reducing rates of stent thrombosis, and the evidence highlights that it is the premature discontinuation of antiplatelet therapy that is the most important predictor for stent thrombosis (Chieffo et al, 2004; Ong et al, 2005). The other key predictors for stent thrombosis development are the patient type, comorbidity of renal failure, bifurcation lesion, diabetes and low ejection fraction. Future technology has focused on developing a more corrective antiplatelet strategy, with better selection of patients. However, the role of the stent type cannot be understated – with certain types; the drug-eluting stents, indicating a greater propensity for stent thrombosis occurrence. Stent thrombosis is a significant concern despite its relatively low incidence (1.3% in this study and previous trials place the rate at 0.4% with Sirolimus and 0.6% with Paclitaxel) due to the widespread use of drug-eluting stents as PCI in coronary artery disease patients. This is further exacerbated by the clinical consequence of stent thrombosis, which is often very severe with a case-fatality rate of 45% (Lakovou et al, 2005).

1.5.2.1 Mechanism of Thrombosis formation

The mechanism of stent thrombosis formation is multifactorial and includes delayed endothelialisation and healing of the stent-arterial wall interface, late stent malapposition, resistance to antiplatelet therapy, and most importantly discontinuation of anitplatelet therapy (Tsimikas, 2006; Colombo et al, 2003). The agents released from drug-eluting stents have been shown to not only inhibit smooth muscle cell proliferation, but also inhibit the growth of endothelial

cells at the interface, hence, preventing the natural beneficial healing process taking place (Joner, 2006; Nordmann, 2006). Clinical data comparing drug-eluting stent use to that of bare metal stent, have shown a distinct pattern of late stent thrombosis (up to 4 years), were the window of thrombotic risk is greater and longer for drug-eluting stents then for bare metal stents (Kedia & Lee, 2007). Certain measures have been attempted to reduce the incidence of stent thrombosis, such as the improvements to antiplatelet regimes and the development of newer generation drug-eluting stents, with a focus on biocompatible surfaces which promote the endothelialisation of the surface whilst preventing restenosis and neointima growth (Garg & Serruys, 2010). An autopsy study performed by Joner et el highlighted a potential difference in stent thrombosis rate between drug-eluting stents and bare metal stents with a greater rate of stent thrombosis presented in drug-eluting stent samples, with the principle cause being attributed to delayed arterial healing (Joner, 2006). The incidence of stent thrombosis with drug-eluting stents has been shown to occur in a range of 0.35-3.1% (Kuchulakanti et al, 2006; Moreno et al, 2005; Ong et al, 2005; Bavry et al, 2005). A Meta analysis performed by Nordmann et al demonstrated the trend of increased mortality with drug-eluting stents when compared to bare metal stent after a long follow up of 3 to 4 years. One of the major contributory factors to the increased mortality rate was the development of late stent thrombosis (Nordmann, 2006). A European study tracked the incidence of stent thrombosis in bare metal stent and drug-eluting stent patients over 3 years. 8000 patients were recruited to the study, which demonstrated a linear increase in rates of thrombosis with the drug-eluting stent group at 0.6% per year after the first 30 days of implantation, when compared to the bare metal stent group (Wenaweser, 2006). It may be concluded from the above studies that the risk of thrombosis and related cardiovascular events are higher with the use of drug-eluting stent therapy than with bare metal stents. Aside from issues of antiplatelet therapy, the stent device in terms of biocompatible coating, drug release dosages and drug type are relevant to the risk of thrombosis. Despite an overall mild incidence of risk of thrombosis, the severe nature of the event supports measures to remove this risk from patients.

1.5.3 Limitations of Drug-eluting stents

Percutaneous coronary intervention now outnumbers CABG by more than 3:1 for the treatment of coronary heart disease (Morton et al, 2007). Despite this high level of usage of drug-eluting stents, a number of problems mentioned still remain. Despite in-stent restenosis rates being reduced with the introduction of drug-eluting stents, there still remains a certain percentage of patients suffering from stenosis development. The risk of thrombosis is the major problem with drug-eluting stent use, and has led to a rethink of their use in a number of patient groups. Finally, through the inflammatory and pro-thrombotic nature of drug-eluting stents, there are certain subsets of patients the therapy is not

suitable. Patients with bleeding complications may be avoided due to the need for long term antiplatelet therapy following implantation. Added to all of these, the extent to which drug-eluting stents are suitable for 'real' life clinical situations is debated. These include cases of complex lesions and patients with comorbidities.

1.6 Conducting Polymers

It has been proposed that alongside work on developing the current commercially available drug-eluting stents, it may be beneficial to introduce novel technologies to the stent devices. Conducting polymer (CP) coatings for bare metal stents have recently been suggested as novel coatings for the development of such devices. It is argued that the biocompatibility offered by these polymer coatings supports their use in preventing the inflammatory reaction that leads to stent thrombosis and in stent restenosis (Okner et al 2007). Alongside this, it has been proposed that these conducting polymers can provide an enhanced drug delivery system to that currently offered by commercial drug-eluting stents. The aim of investigating this technology is to establish whether it may provide solutions to the problems discovered in current drug-eluting stents and provide other beneficial properties in percutaneous coronary intervention procedures. A short background on the conducting polymer technology with information describing their key properties and applications has therefore been presented to establish the scientific basis for their potential benefits. Following on from this, consideration will be given to current studies which have examined the biocompatibility aspects of conducting polymers. Finally, a discussion on how their properties and the coating process may be used to provide optimised stent-based drug delivery systems will be presented.

1.6.1 Electropolymerisation method

The development and use of Conducting Polymers dates back to the mid 1970's. They were produced as a novel generation of organic materials that had both electrical and optical properties similar to those of metals and inorganic semiconductors, but which also exhibited the attractive properties associated with conventional polymers such as ease of synthesis and flexibility in processing (Heeger, 2002). Conducting polymers can either be synthesised by chemical or electrochemical methods. The electrochemical method provides certain advantages that include the ability to generate thin films, ease of synthesis process and entrapment of molecules within the polymer (Guimard et al, 2007). Currently, electrochemical polymerisation is commonly performed using a three-electrode configuration; a working, counter and reference electrode are immersed in an appropriate solvent containing the monomer units and a dopant/anion. Current is passed through the solution via the electrodes, promoting electrodeposition at the working electrode (or anode).

The monomers at the working electrode surface undergo oxidation to form radical cations, these bond to form insoluble polymer chains on the electrode surface. All conducting polymers can be synthesised chemically, however, synthesis by the electrochemical route is limited to those monomers that can be oxidised in the presence of a potential to form the reactive radical cation intermediates necessary for polymerisation. The standard conducting polymers: Polypyrrole, Polythiophene, Polyaniline and PEDOT, can be produced by both chemical and electrochemical methods (Guimard et al, 2007). As mentioned, the polymerisation reaction proceeds via the development of radical cation intermediates. Generally the stages involve the initial oxidation of the monomer units, followed by a coupling reaction involving deprotonation of the couple and a one-electron oxidation of the pair to regenerate the aromatic system (Skotheim, 1986). Polypyrrole is the one conducting polymer that has been studied the most extensively. The following discussion will therefore focus on pyrrole. Its production method, material properties and potential applications will be considered, with much of the discussion directly applicable to many other conducting polymers. A schematic drawing of the events in the polymerisation process is represented in Figure 1.

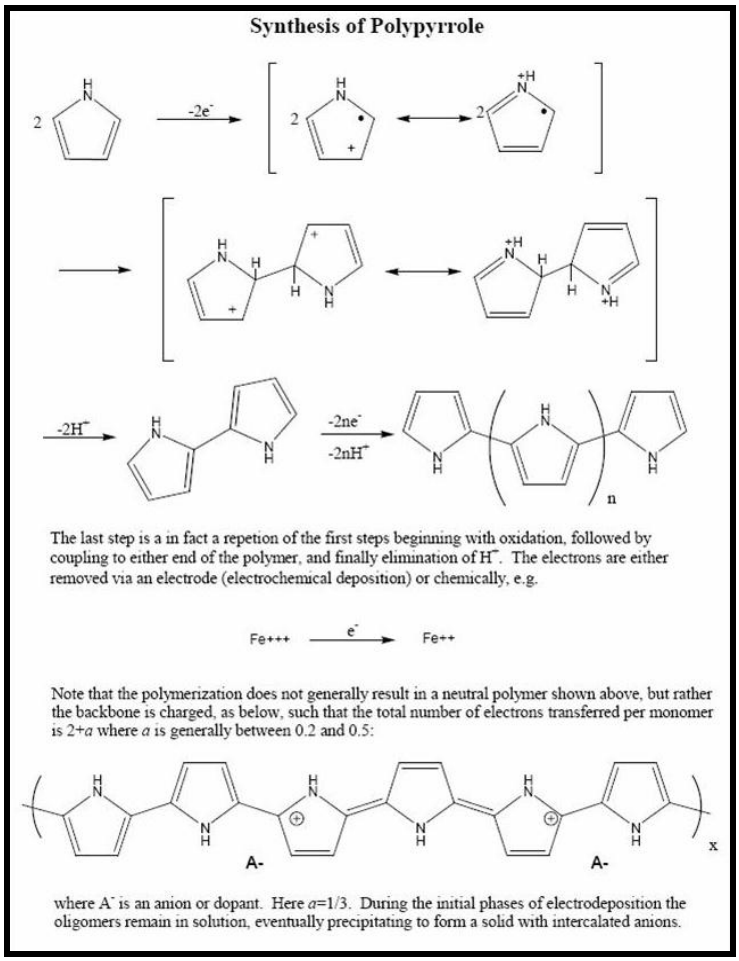

Figure 1.1 Schematic representation for the mechanism of Polypyrrole polymerisation. Source UBC Engineering Mechanical Mechatronics Notes.

The key first stage in the polymerisation process of Pyrrole is the oxidation of the individual monomer units. This process requires a potential in the system and a working electrode which would accept the donation of an electron. The key requirement of the electrode material is that it will remain inert under the conditions of the process, such that the metal itself does not competitively oxidise in place of the monomer units (Skotheim, 1986). Hence, materials such as graphite or stainless steel are usually chosen as the metal working electrode. The potentials generated at the electrode should be sufficient to oxidise the monomer units and resultant dimer (monomer pairs) that are generated. The major reaction proceeds via this formation of radical cations of the monomer, which go on to react with a 2nd radical cation to form dimer units, following the elimination of 2 protons. If the necessary potential is available to oxidise these dimer units, then these units may react with other radical cations to further build up the Pyrrole chain (Diaz & Kanazawa, 1981). The continued chain growth will be terminated in either of two circumstances: the point at which the radical cation of the growing chain becomes unreactive or when the reactive end of the chain becomes sterically blocked from further reactions (Street et al, 1983). The final polymer chain will bear a charge of unity for every 3 to 4 Pyrrole rings, with these charges being counterbalanced by the anion found in the solution. Although the level of polymerisation in the aromatic rings is unknown for these polymers, estimates suggest that they could be as low as 10 or as high as 100 aromatic rings per chain (Natta et al, 1958). This provides an estimated chain of 100 to 1000 Pyrrole units, with an equivalent estimate of 25 to 250 anion molecules respectively (Diaz, 1981). In the conditions presented, the electrochemical polymerisation of Pyrrole has been reported as being a fast process, occurring a few seconds after the beginning of the anodic current flow with the electrode becoming coated with the polymeric film.

The conducting polymers have had prominence for many years for their semiconductor like properties and applications. However, it was only recently that their use in a biomedical setting has been investigated (Guimard et al, 2007). Alongside conductivity, the polymer coatings may offer a biocompatible surface, the potential to harbour biological molecules and surfaces that can be supportive or restrictive to cell adhesion. The variability in mechanical properties including strength and malleability has further increased the number of applications. The interest in the development of conducting polymers stems from the ease by which the aforementioned properties can be altered by varying the experimental conditions. These conditions include the electrode system, amount of charge, charge time, charge type, temperature of synthesis, anion choice and solvent system (water content, pH, concentration of monomer and anion).

1.6.2 Conducting Polymer Properties

The major property of conducting polymers is their ability to have conductivity, this involves being able to receive and provide electrical charge. The relevance of this property in bioengineering applications stems from the affects the surface charge may have on cell adhesion and viability on the surfaces. The electrical or conductive capacities of conducting polymer arise from the method of polymerisation. Following the polymer chain production, the polymer is expected to hold a net charge of zero, due to the close association of the counter ions (doped anions) with the charged conducting polymer backbone. This process introduces charge carriers in the form of charged radical ions into the polymer. The attraction of electrons in one repeat unit to the nuclei in the neighbouring unit yields charge mobility along and between the chains and this is referred to as 'electron hopping' (Guimard et al, 2007). The ordered movement of these charge carriers along the conjugated conducting polymer backbone produces electrical conductivity. Experiments have shown that conducting polymers with conductivities ranging from 0.0001 to 9 S/m have been found to enhance cell growth, supporting the biocompatible capacity of these coatings (Cui et al, 2001). Through these means, the use of conducting polymers in tissue engineering applications has been investigated. It is proposed that scaffolds or devices coated with conducting polymers may be conducive to cellular growth and development. The general properties of conducting polymers such as conductivity, reversible oxidation, redox stability, biocompatibility, hydrophobicity, three-dimensional geometry and surface topography are all desirable in the context of the aforementioned application. Polypyrrole coatings have been reported in previous in-vitro studies to support cell adhesion and growth of a number of different cell types, including endothelial cells (Garner et al, 1999; Garner et al, 1999b). As mentioned, the surface properties of the conducting polymer may also play a key role in mediating cell attachment. This property is dependent on the experimental conditions chosen to produce the polymer layer. The surface roughness of a conducting polymer film can be tailored by modifying the electrochemical synthesis temperature. By increasing the roughness, cell attachment may be favoured (Svennersten et al, 2009). The mechanical properties of the film itself, including its strength, brittleness or malleability are all variable and dependent on the experimental conditions chosen, namely the choice of solvent and anion. The anions chosen are known to influence the structural properties of the film, affecting aspects such as strength and elasticity. One study involved doping Pyrrole with 3 different anions: toleunesulphonate, percholorate, fluoroborate to determine the effects of dopant selection on film properties. The study demonstrated that the use of toleuneosulphonate produced films which were stronger and had higher tensile stretch then the other films (Skotheim, 1986).

1.6.3 Experimental Parameters

The experimental conditions of the polymerisation process that have most marked effect on the final properties of the polymer are the choice of monomer and its concentration, choice of solvent, choice of anion and the potential applied. The role of the solvent used to support the process has shown to be crucial through its influence over the oxidation reactions taking place. Generally, experiments have shown that a solvent with poor nucleophilic characteristics supports the polymerisation process (Diaz et al, 1981). One experiment worked on producing Polypyrroletoluenosulphonate films in acetonitrile solvent with varying degrees of water (Banks & Bailey, 1964). Water is generally known to be a poor nucleophile, hence, its use as a solvent providing an aqueous medium for the monomer and anions. The addition of water to the Acetonitrile solvent was shown to produce films with enhanced conductivity and support the elastic potential of the film. The mechanism by which water supports these properties is via its poorly nucleophilic status. As mentioned previously, polymerisation proceeds via the development of radical cation intermediates. This reaction is particularly sensitive to the nucleophilicity of the environment in the region near to the electrodes – where the radical cations are generated. The poor nucleophillic status ensures that the solvent does not react with the monomer units but rather polymerisation on the electrode surface is supported. The solvent around the electrode is crucial in ensuring that the oxidation reactions favour polymerisation chain growth rather than oligomer formation (chain of monomers) which is often an unwanted effect of chemical polymerisation taking place at the same time. Hence, it has been shown that as water is added, the negative order dependence of the chemical polymerisation rate favours electrochemical polymerisation, hindering the chemical generation of oligomers (Downward & Pletcher, 1986). Therefore, as water is added to a solvent like acetonitrile, the electrical and mechanical properties of the Polypyrrole are strongly improved. However, one limitation of having water within the solvent is that the oxygen in water may selectively target the reactive radical cations in the polymeric chain leading to degradation over time. The oxygen does this through its nucelophilic properties which are normally controlled by the hydrogen ions of water (Asavapiriyanont et al, 1984). This reaction is said to be minimised if changes are made to increase either the monomer concentration or the electrolyte concentration or if the synthesis temperature is reduced. An acidic environment within the solvent/solution has also been shown to support the polymerisation process (Nalwa, 1997). For polymerisation to proceed, protonation of the Pyrrole needs to occur. The protonation supports the reaction by donating an electron to the metal electrode and supporting intermediate radical cation formation. Generally, protonated Pyrrole molecules are not aromatic and therefore, more easily oxidised. Hence, by acidifying the aqueous solution and supporting the increased rate of the initial protonation of Pyrrole, the polymerisation rate may be increased.

1.6.3.1 Anion

The anion/dopant used in the solution is known to affect the rate of polymerisation and the properties of the resultant film. The key component of any chemical chosen for the role is that it assumes a net negative charge when in solution. The amount of anion that is found in the film is governed by the level of oxidation of the polymer. Studies have shown the Polypyrrole oxidation level to be at a 0.25-0.35 ratio per unit, which implies an anion content of 25 – 30 % by weight (Mui & Grunwald, 1982). This corresponds to one anion for every 3-4 units. As with the solvent, anions that are poorly nucleophilic permit the formation of good quality films. The level of oxidation that occurs is described as an intrinsic characteristic of the polymer and is described as being unaffected by the nature of the anion. However, the anion is said to influence both the mechanical and electrical properties of the film. As previously mentioned, the use of toleunesulphonate was shown to produce Polypyrrole films with greater tensile strength then those produced using perchlorate or fluroborate as an anion (Skotheim, 1986). The main qualities for a suitable anion are the solubility of its salt, degree of dissociation in solution and its reactivity (Nalwa, 1997). The surface morphology may also be affected by the choice of the anion. The anion concentration has an effect on the polymerisation process, with increased concentrations leading to more rapid rates of polymerisation. This is supported by the view that the anions generally stabilise the growing radical cations, favouring coupling reactions rather than unwanted secondary chemical reactions (Diaz & Kanazawa, 1981).

1.6.3.2 Potential

The potential delivered to the electropolymerisation system is known to have an effect on the properties of the conducting polymer, including film thickness, surface morphology, mechanical properties and conductivity (Skotheim, 1986). The potential may be varied by method of application, amount and time duration of delivery. The potential may be delivered in a potentiostatic manner, by cyclic voltammetry or in galvanostatic conditions. Each method would respectively affect the level of oxidation taking place at the working electrode. Potentiostatic involves delivering a constant electrode potential for the duration it is applied. Cyclic voltammetry involves the potential being increased with time and at a set point the potential delivered is inverted. Galvanostatic conditions involve delivering a constant current throughout the procedure. Generally it has been shown that with an increased delivery potential an increased polymer weight gain is achieved (Genies et al, 1983). This process has a peak range at around 1.8 to 2.2 V depending on the water content in the solvent. After this peak, the polymer weight able to be generated begins to fall and eventually falls towards zero. Hence, a viable zone in which polymerisation would occur has been reported as being between 0.8 to 2.9 volts (Genies et al, 1983). The reason for the peak voltage performance and a fall

in production above this is attributed to the previously described competition between desired electrochemical polymerisation of Pyrrole and the route of chemical polymerisation. If the second case (chemical) polymerisation is promoted, this would result in incomplete growth of the polymer chain and the deposition of non-conducting oligomers over the electrode surface (Beck et al, 1990). The very high levels of anodic potentials provide a large current flow through the system, producing a local change in pH at the reaction layer, which facilitates the chemical polymerisation process. Hence, polymerisation growth is stunted (Otero & Rodriguez, 1994).

1.6.4 Biomedical Applications of Conducting Polymers

Biomedical applications of conducting polymers include biosensors, tissue engineering scaffolds, neural probes, drug delivery devices and bio-actuators (Guimard et al, 2007). Already mentioned is the potential use in tissue engineering through the ability of the surface to support cellular adhesion. Tissue engineering applications involve the construction or coating of scaffolds using conducting polymer technology. Conducting polymers can be generated with biocompatibility, good conductivity and possible surface modifications that would allow for cell activation to be triggered or the promotion of cell attachment. Depending on the production method selected, conducting polymers may also be produced with optimised biological properties through the incorporation of bioactive molecules. These may be incorporated by physical adsorption onto the polymer surfaces, entrapment within the film, by doping or by covalent attachment (Guimard et al, 2007).

1.6.4.1 Conducting Polymer in Drug Delivery

A major area of interest is the role that conducting polymers may play in the development of enhanced methods of drug delivery. Much of the drug delivery systems developed have focussed on the expulsion of anions/drugs following the reduction of the conducting polymer through electrical stimulation (Hodgson et al, 1996; Li et al, 2005). Several studies have investigated the release of a number of therapeutic agents (including nerve growth factor, dexamethasone, and heparin) from various conducting polymers following electrical stimulation. In the case of nerve growth factor, release was demonstrated following the application of a negative potential to the Polypyrrole coating, resulting in its reduction and a rapid expulsion of anions (nerve growth factor) within less than a minute (Abidian et al, 2006). Similar studies on the attempted controlled release of Dexamethasone from a conducting polymer, following electrical stimulation, resulted in an initial burst release in the first few days, with very little release thereafter (Wadhwa et al, 2006). Only a limited number of studies have been performed which have examinined the elution of drug from a conducting polymer without the need for electrical stimulation. These studies have attempted to

demonstrate the potential elution of agents from the conducting polymer, along with demonstrating a profile of release.

A study focused on entrapping oligodeoxynucleotides (ODN) within a conducting polymer coating (Piro et al, 1999). The aim was to investigate the use of polymer films as reservoirs of biologically active substances for in vivo delivery to target tissues. The conducting polymer used was PEDOT with ODN as the doping anion. The 3 electrode configuration was used, with a cyclic voltammetry approach to applied potential, scanning between -0.1 to 1.35 V. A limit of 20 voltametric cycles was selected as preliminary results demonstrated that more cycles than this produced further polymerisation that led to fragile, friable surface layers that could become detached from the electrodes during handling. The ODNs were radiolabelled and analysis demonstrated that they were able to diffuse deep within the polymer matrix, rather than the presumed simple adsorption onto the polymer surface. Also, they were able to show that by increasing ODN concentration within the solution they were able to produce films with greater uptake of the ODNs. The produced films were immersed in phosphate buffer solution to analyse the rate of ODN elution. The profile of release obtained was characterised by three distinctive sections. Initially, a stage of early burst, followed by an intermediate release period and finally ending in a period of slow release. It was presumed that the burst stage represented the fast dissociation of ODN species that were electrostatically bound to the film surface. The slowing down of this initial release then represented the picture of a diffusion rate from the peripheral layers of the film. The final slow part is indicative of a typical diffusion controlled release from locations in the bulk of the material. The authors proposed that simple diffusion may be taking place down concentration gradients but also that a certain level of ionic exchange also takes place. The incoming anions are said to contribute to the increasing ionic strength of the interior medium and at the same time inducing the dissociation of the ODN-polymer complexes (Piro et al, 1999).

A study developed further interest in the versatile aspects of the Polypyrrole production methods by proposing specific additions to the side chains of Pyrrole (Weiss et al, 2003). Alongside the normal polymerisation generation, functional groups may be added to the side chains of the Pyrrole, mostly in the N or 3 positions, to elicit changes in the film's mechanical property. This work highlighted how the stability and composition of the coatings could be advanced by the addition of certain monomers to these side chains. The monomers added to the N position produced films that were shown to be symmetrical and regularly structured. Addition of monomers on the 3 position produced irregular structures. Their findings highlighted the versatility in Polypyrrole production and the positive developments in the polymer properties as a result of these changes. They proposed that future work would focus on the incorporation of bioactive agents into the film coating by chemical

conjugation to the Pyrrole derivatives (namely by covalent bonding) or embedding the molecules into the coating during or after electropolymerisation coating (Weiss et al, 2003).

A further study attempted to achieve the attachment of a biomolecule of interest, hyaluronic acid, to the Polypyrrole chain by a number of different methods. In one method, hyaluronic acid was covalently bonded to the Pyrrole monomers before performing electropolymerisation; the aim was to compare the results of this method to the binding of hyaluronic acid to Polypyrrole via the doping approach. It should be noted that a conducting polymer coating/film that had the hyaluronic acid embedded and fixed for its duration of use was desired. The observation of hyaluronic acid release following doping was an undesirable outcome of the study. The study was able to demonstrate that hyaluronic acid covalently bonded to Pyrrole, rather than doped, was more unlikely to be released from the polymer surface. Experiments on doped hyaluronic acid have demonstrated release of the molecules from the conducting polymer over time. This release has been attributed to the dual effects of dedoping and as previously mentioned a dopant exchange. Interestingly, the study adds a future perspective on surface modification techniques in conducting polymer development. They argue that these techniques could be used to develop more hydrophilic surfaces that would improve conducting polymer biocompatibility, by preventing the adsorption of plasma onto the surface, which is known to lead to the classic inflammatory response. These surface modification approaches may have a role in biocompatible coating developments in stents – either supporting growth of endothelial cells for healing or preventing the adsorption of cells that lead to an inflammatory response (Lee & Schmidt, 2010).

1.6.4.2 Conducting Polymers in Stent based Drug Delivery

Following on from the application of conducting polymers in a drug delivery role, it was proposed that this mechanism could be utilised in the function of a drug-eluting stent. Therefore, a number of studies have been performed to analyse the effectiveness of conducting polymers as drug-eluting stents.

A study performed by Arbizzani et al attempted to produce conducting polymer films on platinum wires, that would elute selected drugs over a time period, as a precursor to the use of such technology in a drug-eluting stent application. Different Polypyrrole coatings were produced by doping with toleunosulphonate, salicylate and naproxene, all separately. The electropolymerisation procedure was performed at room temperature, with a Pyrrole concentration of 0.1 M. The study was able to demonstrate the ability of Polypyrrole to become doped by the aforementioned anions. Following on, immersion studies demonstrated the ability of the Polypyrrole coatings to release these drugs over a period of days. The toleunosulphonate and naproxene were eluted in total over

30 days. Interestingly, the data from the salicylate release showed that this drug was eluted rapidly and completely by 6 days. Arbizzani's team promoted their positive findings as highlighting the Polypyrrole's capacity to become doped by and subsequently elute an array of agents (Arbizzani et al, 2007).

One study attempted to observe the effects of producing multiple layer drug-eluting stent coatings using conducting polymer technology to improve the functional properties and to support a more desired release profile. In this particular study, three types of stent coatings were produced, (i) a simple complex of Paclitaxel with Pyrrole on a bare metal stent, (ii) the previous matrix but bound onto a primer coating, and (iii) employing a primer coating beneath and top coat covering the simple complex film. The 3rd coating type described, is analogous to the system used in the commercial Paclitaxel eluting stents. In the 1st type of coating, 55% of the drug was shown to be released within the first 3 hours – a similar picture is shown for the 2nd type. The authors offer that this may represent the Paclitaxel that is found on the surface and hence, the primer coating would play no role in altering the initial release. However, over the following 30 days, 20% less drug is released from the 2nd coating vs the 1st. They attribute this finding to the non uniform and unstable nature of the 1st coating; because of its to attachment to the metal stent, which accelerates the Paclitaxel release. In the 3rd coating formula, which contained a covering primer layer, the burst release was minimised to 20% and the release rate also reduced. This resulted in 50% less of the Paclitaxel being released when compared to the 1st coating. This highlights the value and role of the thin top layer in promoting a diffusion controlled system of release (Okner et al, 2009).

1.6.4.3 Limitations of existing approaches to Conducting Polymer in Drug-eluting stents

The above studies of conducting polymers as drug releasing agents and as drug-eluting stents provide us with a greater insight into how the anion/dopant/agent of interest may be attached or embedded into the conducting polymer coating. Possible mechanisms by which this molecule may be eluted over time were also tested and discussed. The studies demonstrate that conducting polymer coatings have the potential to act as drug-eluting stents. However, only a limited number of the studies attempted to compare the agent release profile by adjusting coating preparation conditions. It seems very relevant to examine which electropolymerisation experimental conditions would provide more beneficial properties in terms of coating quality and drug release profile from these coatings. Thus, there is the need to better understand how the process parameters used in the electropolymerisation process affect subsequent drug release. This will provide an opportunity to optimise experimental conditions, such that advanced novel bare metal stent coatings may be

produced, which if successful will provide substantial improvements to the treatment of coronary heart disease. The release profiles obtained by these previous studies fall short of the desired aim of drug-eluting stents, which involves a gradual release over 30 days. Hence, it is desired to obtain drug release profiles that display such a release. The aforementioned experiments also only focus on conducting polymers as applied to a piece of wire rather than the true application of a coronary stent. Hence, studies testing the use of conducting polymers in their intended application are necessary.

1.7 Project Aims

The overall aim of this study is to investigate the capacity of conducting polymers to form films of desired property over stainless steel wires and actual bare metal stents. Further, it is intended to test the capacity to store and elute a relevant pharmaceutical agent over a 30 day period. An investigation into whether the release profile of the drug may be optimised by varying particular production parameters of the electropolymerisation process will form the conclusion of the study. Specific aims of the project are included below:

- Coat stainless steel wires with a conducting polymer using the electropolymerisation method and a monomer of Pyrrole and a dopant of Salicylate
- Coat stainless steel wires with a conducting polymer using the electropolymerisation method and an array of different Pyrrole and Salicylate concentrations
- Analyse and observe the mechanical properties of the produced coatings.
- Measure Salicylate release from produced stainless steel coatings over a 30 day period
- Coat bare metal stents with a conducting polymer using the electropolymerisation method and optimum Pyrrole and Salicylate concentrations based on above findings
- Analyse and observe the mechanical properties of the produced bare metal stent coatings when left crimped and when expanded
- Measure Salicylate release from produced bare metal stent coatings (left crimped and expanded) over a 30 day period.

CHAPTER TWO

METHOD

2.0 Introduction

This chapter covers the methods used to produce the conducting polymer coatings and those to assess the elution of drug. It will include the materials necessary for the experiment, the method design for the electropolymerisation coating production part of the procedure, the calculations for solvent and drug preparation and the means by which drug release measurement was performed. The experimental work involved preparing conducting polymer coatings on stainless steel wires (with the entrapment of a drug) and analysing/measuring the elution amount and rate of the drug release over a 30 day period. This work involved three major parts, (i) the production of stainless steel wire coatings, (ii) immersion of coated wires in a physiological solution, (iii) performing absorbance measurements to analyse the amount of drug eluted into the solution.

2.1 Materials

2.1.1 Chemicals

Sodium Salicylate and Pyrrole were purchased from Sigma-Aldrich, Dorset, UK. All chemicals were used as supplied unless stated otherwise, and were stored according to the specific requirements detailed in the relevant material safety data sheet.

2.1.2 Stainless Steel Wires

In the main series of experiments, the polypyrrole coatings were formed on stainless steel wires rather than bare metal stents. The stents often used in clinical practice are stainless steel 316L in type; hence, by using stainless steel wire we would be able to replicate the nature of the surface on which the coating needs to develop. Stainless steel wire 316L (5 m length, 1 mm diameter) was purchased from Goodfellow-Cambridge (Huntingdon, England).

2.1.3 Coronary Stents

Two coronary stent systems were kindly donated to the project by Prof Keith Oldroyd (West of Scotland Heart and Lung Centre, Glasgow, Scotland). These stents were bare metal Liberte^{TM} Coronary Stents (Boston Scientific, MA, USA).

2.1.4 Electrodes

A three-cell electrode design was used with a stainless steel wire 316L (5m length, 1mm diameter) purchased from Goodfellow-Cambridge (Huntingdon, England) acting as the working electrode material. Platinum wire (1mm diameter) purchased from Goodfellow-Cambridge (Huntingdon, England) acted as the counter electrode material. A KR5 reference electrode purchased from ThermoScientific UK Ltd, Leicestershire, England acted as the reference electrode.

2.1.5 Instrumentation

Electropolymerisation was performed by utilising a Solartron SI1287 electrochemical interface. The analysis of drug elution was performed on a UV-vis spectrophotometer.

2.2 Polypyrrole Coating development

2.2.1 Electropolymerisation design

As mentioned in the introduction chapter, one of the methods most widely used to produce conducting polymers is electropolymerisation. A three-electrode cell was utilised to perform electropolymerisation in the present study (Figure 2.1). This consisted of a stainless steel wire taking the position of the working electrode, a platinum wire acting as the counter electrode and a standard reference electrode (KR5). The latter two electrode materials were chosen because of their demonstrated use in the literature and successful experience of their use within the laboratory in which the present study was carried out. The electropolymerisation unit, SolartronSI1287 interfaced to a desktop computer with Corrware software, this was used to adjust the experimental conditions of electropolymerisation type, voltage level, and duration of application. Potentiostatic electropolymerisation, were the voltage applied is in one direction and constant, was selected to produce the coatings. This method supports the electropolymerisation process occurring along the oxidation route and hence the doping process of the negatively charged pharmaceutical agent, Salicylate. Based on previous findings in studies comparing voltage level to resultant film thickness, mechanical properties and conductivity, and results from preliminary work carried out previously within the laboratory, a constant applied voltage of 1.3 volts vs reference was selected for use in all electropolymerisation procedures. Again, the time over which the voltage was applied was decided upon by the combined findings of previous experimental work, work performed in our laboratory and results from preliminary experiments. In addition, given that future development of the

technology may involve manufacture scale production or patient-specific production within the hospital catherisation suite, it was considered that 10 minutes would be a suitable time frame for such potential applications.

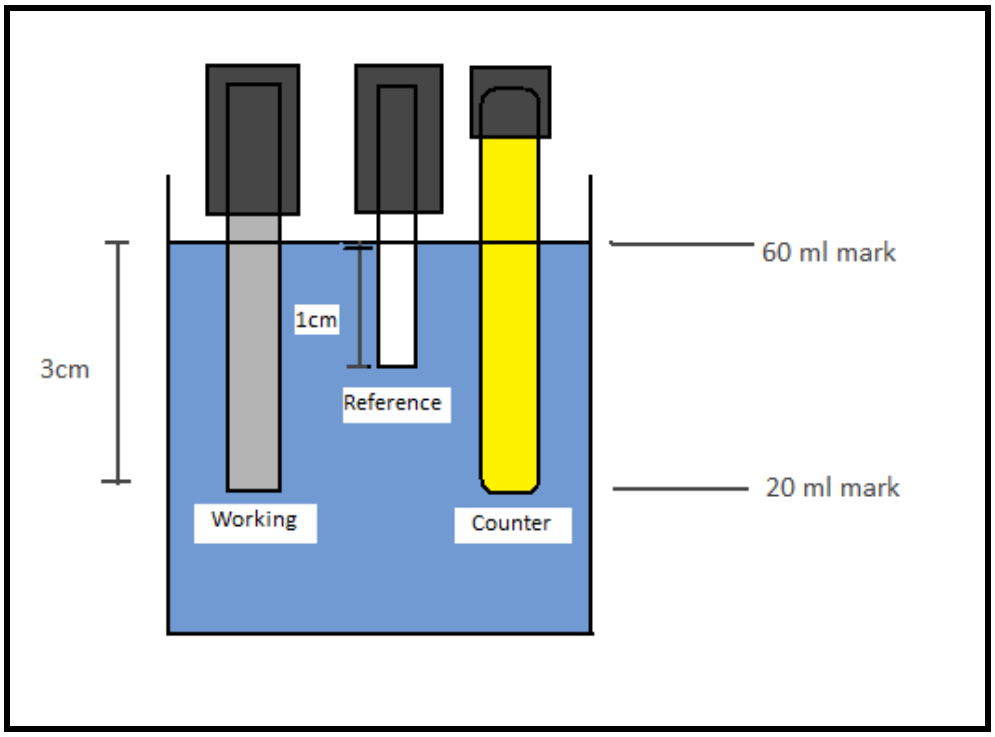

Figure 2.1 Configuration of 3 compartment cell used during Electropolymerisation experiments. The figure shows the position of the working, reference and counter electrode within the solution.

2.2.2 Coating Solution Selection

The solution in which the 3 electrodes were immersed consisted of an aqueous medium (distilled water), the monomer (Pyrrole) and a dopant (Salicylate). As mentioned in the section 1.6.3 of the introduction, a number of solutions, such as acetonitrile and water, have been utilised in the past due to their effectiveness in producing films with strong mechanical and electrical properties. Water was selected as the aqueous medium in the present study because it has been shown to exhibit the desired property of having a poor nucleophilic status (Banks & Bailey, 1964). Consequently, water has been shown to promote the electropolymerisation process in place of undesired secondary chemical reactions (Nalwa, 1997). Pyrrole (Py, Aldrich, 98%) was selected as the monomer to be

studied since it has demonstrated consistent results across a variety of biomedical applications, including in drug-eluting stents (Guimard et al, 2007).

2.2.2.1 Dopant Anion choice

The anion plays a key role in the electropolymerisation process by binding within the polymer chain, which provides electroneutrality to the chain (Diaz & Kanazawa, 1981). Experimental work has proposed a binding ratio of approximately 1 anion to every 4 Pyrrole monomers (Natta et al, 1958). Further work has shown that the charge nature of the electrolyte and its unique properties have substantial effects on the film/coating properties (Street et al, 1983). Finally, in controlled drug delivery applications, a pharmacologically active anion can be used to not only support the polymerisation coating process but also to produce a drug-polymer matrix bound to the metal surface, with drug elution from the coating occurring over an extended time period. Sodium Salicylate (NaSa, Sigma, 99.5%) was chosen for a number of reasons, including its solubility in aqueous medium, ability to dissociate into an anion and previous work verifying its ability to support the polymerisation process and undergo elution over time (Arbizzani et al, 2007). Another major reason for its inclusion is that it exerts a series of potentially therapeutic biological effects. Since this study sought to investigate the possibility of using conducting polymers as drug-eluting stents, it was of paramount importance that it can be shown that a relevant pharmaceutical agent may be entrapped and eluted from the coating. Salicylate, being from the family of Aspirin agents (acetylsalicylic acid), is primarily recognised as an anti-inflammatory agent. Salicylate acts by inhibiting prostaglandin synthesis (Mackowiak, 2000). Some of the functions of prostaglandins include the regulation of platelet aggregation, control on inflammation and control on vascular permeability (Rang, 2003). As mentioned previously, a key component that leads to the development of in stent restenosis and stent thrombosis following stent implantation is the inflammatory reaction (Farb et al, 1999).

Hence, it is proposed that with the administration of an anti-inflammatory agent at the drug-eluting stent site, the inflammatory reactions that normally occur may be avoided. This therapeutic effect, at least in theory, may act in addition to the biocompatible support afforded to the stent by the conducting polymer coating, and therefore has the potential to improve outcomes following stenting. To summarise, the inclusion of Salicylate as an anion was based on its ability to serve the dual roles of supporting the process of film formation and for a potentially therapeutic effect following elution from the film coating. Our 3 cell compartment configuration was therefore made up of a stainless steel wire working electrode (to mimic a standard coronary stent), platinum wire counter electrode and standard KR5 reference electrode. The solution in which these would sit was

made up of a combination of a water aqueous medium with varying concentrations of Pyrrole and Salicylate.

2.2.3 Coating Solution Preparation

Preparation of the coating solution involved the collection and amalgamation of the three main components: distilled water, Pyrrole and Sodium Salicylate. The amount of distilled water within the solution batch was always a constant of 250 ml (60 ml of total solution was used for each run of the experiment, 3 runs were done to produce 3 coated wires - made from the same electropolymerisation conditions), whilst the concentration of Pyrrole and Salicylate were varied depending on the experimental conditions selected. The formula for calculating the required amount of Salicylate and Pyrrole concentrations relative to the amount of water is as follows:

Mass (grams) Sodium Salicylate = Number of Moles x Molar Mass

Example Calculation:

For 250 mls of 0.1 M sodium salicylate solution the mass of sodium salicylate required is as follows:

Mass = (0.1 M x 0.25 L) x 160.1 = 4.0025 g

Pyrrole was supplied as a 98% solution. The volume of pyrrole monomer required to achieve a given concentration within the coating solution was calculated as follows:

A 0.1 M of Pyrrole required the addition of 1731.25 µl

A 0.5 M of Pyrrole required the addition of 8656.25 µl

A 1.0 M of Pyrrole required the addition of 17312.5 µl

2.2.3.1 Experimental Parameters

The following solutions were prepared and used as coating solutions in the electropolymerisation process:

0.1 M Pyrrole + 0.1 M Salicylate

0.5 M Pyrrole + 0.1 M Salicylate

1.0 M Pyrrole + 0.1 M Salicylate

0.1 M Pyrrole + 0.5 M Salicylate

0.5 M Pyrrole + 0.5 M Salicylate

1.0 M Pyrrole + 0.5 M Salicylate

0.1 M Pyrrole + 1.0 M Salicylate

0.5 M Pyrrole + 1.0 M Salicylate

1.0 M Pyrrole + 1.0 M Salicylate

2.2.3.2 Solvent preparation

The solution value of 250 ml dH20 was selected to allow for the electropolymerisation solution of 60 ml to be achieved, with a fresh batch of the 60 ml being used in each repeat of the process, 3 runs were performed for each experimental condition. The water was measured out in a conical flask and added to a 500 ml capped beaker. All equipment was rinsed with distilled water before use. Sodium Salicylate was firstly weighed out to its required amount; note Sodium Salicylate is found in solid powder form in the laboratory, and then added to the beaker of distilled water. Safety glasses and gloves were worn through this procedure to protect against the irritant nature of Sodium Salicylate powder. The substances in the beaker were mixed in a standard manner and for a standard amount of time. Pyrrole; found in its liquid form, was added to this beaker solution in a fume cupboard. This was done to protect the investigator from the harmful nature of the Pyrrole vapours. Safety glasses and gloves were also worn. Again this solution was mixed by a standard method and for a standard amount of time. All safety precautions stipulated in the relevant MSDS and COSHH form, were adhered to throughout every experimental protocol.

2.2.4 Electropolymerisation procedure

The electropolymerisation arrangement was set up as described in section 2.2.1. 60 ml of the prepared coating solution was added to a 100 ml beaker selected. The stainless steel wire (1.0 mm diameter) was prepared for use as the working electrode by cutting a standard length of approximately 4.5 cm. The wire section was rinsed in distilled water and the surface dried by a drying towel prior to use. Initially, the wire section was weighed, to allow for a corresponding weight gain to be measured after coating formation, thus indicating change in weight and hence approximate a weight of the film coating produced. A platinum wire was prepared for use as the counter electrode.

Since this electrode was to be used repeatedly, the platinum wire was sanded to remove any residues and rinsed and dried before every use. A standard KR5 reference electrode was used as the reference electrode. It was stored in saturated KCL solution (approximately 3 M) between uses, according to the manufacturer's instructions, and rinsed in distilled water before use. The corresponding leads from the device were connected for working, counter and reference electrodes, with a spring clip attached to the ends of the working and reference electrode. These 3 connecting electrodes were immersed in the prepared 60 ml solution by the aid of clamps and a stand. The configuration in terms of depth of immersion and position of electrodes was maintained as standard for every electropolymerisation experiment. The experimental set up, detailing the three-electrode configuration, is summarised in Figure 2.1.

Once the correct set up had been achieved, the parameters for the electropolymerisation process were entered into the computer interface. A potentiostatic voltage setting of, 1.3 V versus reference was applied for 10 minutes duration. After completion of the coating process, visual inspection of the resultant polymer coated wires were made, notes were recorded on the coating quality achieved, with a particular emphasis on thickness, uniformity and surface roughness. Each stainless steel wire was then removed and left to dry for 24 hours at room temperature. During this time, all coated wires were stored in an inclined position, with the uncoated portion forming the base, so as to prevent the drying surface contacting any other surface. For each experimental condition studied (combination of different Pyrrole and Salicylate concentrations), three replicates were performed. Between each coating, the 'used' solution was removed and a new batch of 60ml coating solution was used each time. The platinum wire was rinsed, sanded and then rinsed and dried between coatings. The standard reference electrode was also rinsed and dried. The previous set up was again performed and the electropolymerisation process performed under the standard voltage conditions aforementioned. This was done 3 times, such that 3 coated wires were produced for each experimental condition. All 3 wires produced were stored next to each other in a vertical position, being left to dry for a 24 hour period.

2.3 Drug release measurement and analysis

2.3.1 Immersion period

After the coated stainless steel wires had been allowed to dry for 24 hours, they were again weighed and the change in weight noted. Subsequently, each wire was then immersed in a 1 ml epindorff tube containing 1 ml physiological solution (0.1 M sodium phosphate (monobasic) buffer solution, pH 7.4). The phosphate buffer was chosen because the salt composition is close to that of blood

plasma. The wires were immersed at room temperature (~ 20 ˚C) in this solution for 10 minutes, 1 hour, 1 day, 3 days, 7 days, 11 days, 14 days, 17 days, 21 days, 25 days and 30 days concurrently following the initial immersion. At each time point, the coated wire was transferred to an epindorff containing 1 ml fresh physiological solution, with the sampled solution then stored at -20 ˚C so that it may be analysed for levels of Salicylate concentration. Prior to storage, observations were recorded for each sample with a focus on the presence and nature of any polymer residues within the release medium. This approach of testing drug elution was decided upon because of its ability to provide an indicator of in vivo drug release and is based on the recommendations of an expert cardiology panel which have been recently reported (Schwartz et al, 2008).

2.3.2 Analysis of Samples

Analysis of the Salicylate concentration in the physiological solution sample was performed on a UV-vis spectrophotometer. This device functions by analysing the level of absorbance of the emitted light passing through the sample. Selection of the specific wavelength at which Salicylate affects absorbance of light was important in ensuring the accuracy and specificity of this measurement device, and an absorbing wavelength of light at 296.5 nm was selected based on a previously published study (Arbizzani et al, 2007). Initially, a calibration curve was generated for a range of standard Salicylate concentrations: $1x10^{-6}$, $1x10^{-5}$, $3x10^{-5}$, $1x10^{-4}$, $3x10^{-4}$, $1x10^{-3}$, $3x10^{-3}$, $1x10^{-2}$, 0.05 and 0.1 M Salicylate. All calibration standards were prepared in the same stock solution of phosphate buffer as used use in drug release experiments (section 2.3.1). Each calibration standard was analysed in the UV-vis spectrophotometer against a standard sample of blank phosphate buffer to obtain a background control absorbance level. From each sample a corrected reading for absorbance specific to the presence of salicylate was therefore obtained. Absorbance values obtained at each standard concentration were plotted against the log concentration of the Salicylate standard to provide a calibration curve. A curve was plotted of Absorbance reading against Log concentration standard of Salicylate. A curve was fitted for these points and a formula generated. Consequently an R squared value was obtained to demonstrate how well the curve (and its formula) described the set of values. R- squared values above 0.9 were only accepted. A typical calibration curve obtained is shown in figure 2.2.

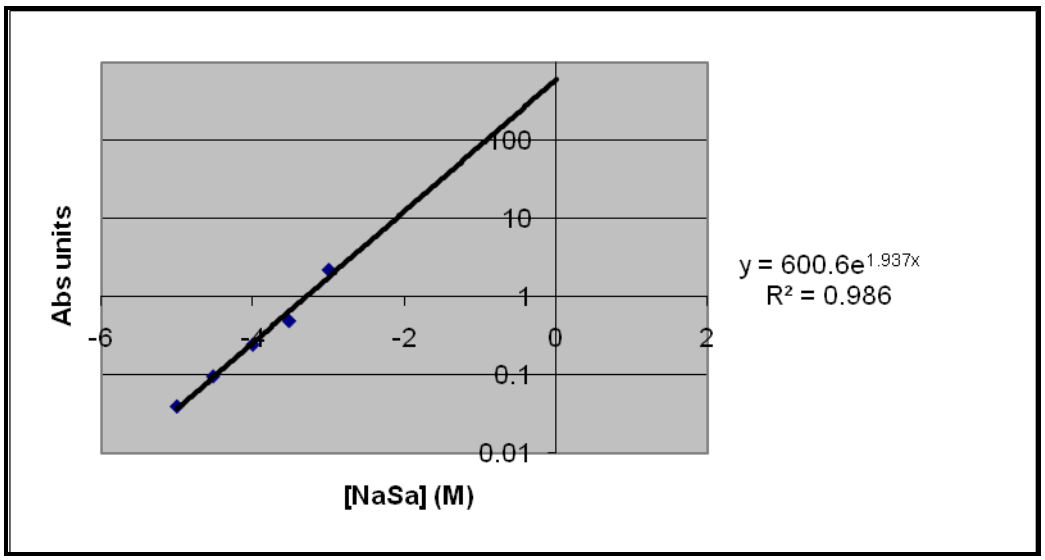

Figure 2.2 Typical calibration curve of Sodium Salicylate concentration against the absorbance value obtained by UV-vis spectrophotometry, with formula for the straight line.

The immersed samples that had been frozen were then allowed to defrost. Sufficient time was provided to ensure all ice particles had disappeared and each sample was then vortexed to ensure even distribution of any Salicylate in the solution. Each sample was then analysed, and an absorbance value obtained, using the same method used for the standards.

2.3.2.1 Calculation of Concentrations

The absorbance reading obtained for each sample was used in the formula obtained from the calibration curve to obtain an estimate for Salicylate concentration.

The value obtained from this calculation would be the log for moles of concentration of the sample. Hence, the calculated value was inversed to provide a value in moles. Often, drug-eluting stent companies quote their stents drug dosage in gram scales, hence, the moles were converted to micrograms.

The above procedure had allowed us to estimate the mass of Salicylate eluted from the coatings as a function of time. In this process, it was crucial that if samples were analysed on a different day (following storage), that a separate calibration curve should be produced for that particular day and group of samples. This was implemented to account for any degradative changes taking place in the preprepared phosphate buffer or Salicylate samples with time. During periods when they were not in

use, the phosphate buffer solution and Salicylate standards were kept in the laboratory refrigerator (~ 4 ˚C).

2.3.3 Drug release profile generation

All data on drug elution measured at the various time points were inputted into standard Microsoft Excel spreadsheets. Drug release profiles were then generated by calculating the cumulative total of drug eluted from the stent coating at each time point. From this data, the drug release profile graphs of combined drug release against time period were generated and analysed.

2.3.4 Analysis comparisons

Different sets of coating conditions were analysed to describe patterns between monomer concentration, anion concentration, the balance of the two and their effect on coating thickness, structural property, mechanical property and drug release profile. The combinations of experimental conditions that were compared for changes in the aforementioned properties are displayed:

- Analysis 1:

  0.1 M vs 0.5 M vs 1.0 M Pyrrole supported by 0.1 M Salicylate

- Analysis 2:

  0.1 M vs 0.5 M vs 1.0 M Pyrrole supported by 0.5 M Salicylate

- Analysis 3:

  0.1 M vs 0.5 M vs 1.0 M Pyrrole supported by 1.0 M Salicylate

- Analysis 4:

  0.1 M vs 0.5 M vs 1.0 M Salicylate in 0.1 M Pyrrole

- Analysis 5:

  0.1 M vs 0.5 M vs 1.0 M Salicylate in 0.5 M Pyrrole

- Analysis 6:

  0.1 M vs 0.5 M vs 1.0 M Salicylate in 1.0 M Pyrrole

2.4 Coating of Coronary stent

Data collected during the experiments on stainless steel wires were used to select a set of coating conditions that would produce supposed optimum drug release whilst retaining the integrity of the coating surface. This method was then used to coat two standard stainless steel bare metal stents. The coating solution that was selected was the 0.5 M Pyrrole and 1.0 M Salicylate mixture. This was based on preliminary evidence showing that these conditions produced a strong coating with high drug elution rates. Two bare metal stents (Liberte^{TM}), which were precrimped onto the inflation balloon, were coated using this solution under the standardised processes for electropolymerisation as described in section 2.2.4. Once each bare metal stent was coated, they were left to dry for 24 hours. After 24 hours, one of the coated bare metal stents was expanded by balloon inflation, while the other bare metal stent was left in its original crimped state. As before, notes were made of the coating thickness on the bare metal stents, along with the response of the coating to being inflated. Following this, the same procedure of immersion and UV-vis spectrophotometer of solution samples were performed to generate absorbance readings over a 30 day period of release measurement. From this data, drug release profiles were generated for the crimped stent and the expanded stent. As before, notes on the structural integrity of both stents were made following their period of immersion in physiological solution.

The results of coating thickness, structural properties, mechanical properties and drug release profile for the crimped and expanded stents were compared between themselves and that of the findings from the stainless steel wire coated with 0.5 M Pyrrole and 1.0 M Salicylate.

2.5 Data Analysis and Statistical Treatment

Unless stated otherwise, all data presented in Tables represent the mean ± one standard deviation, based on a sample size of 3 replicates. Graphs of drug release versus time show the results of single observations as it is believed that this provides a true reflection of the variability in the data sets collected. A large data set has been gathered and a series of comparisons has been made to determine the effect of dopant ion concentration, and pyrrole monomer concentration, on the drug release profiles and coating characteristics achieved. However, given the relatively high variability observed within replicate samples, and the low number of replicates used, it was decided that meaningful statistical comparisons between data sets would be of limited value and for this reason, no further statistical treatment of the data has been performed.

CHAPTER THREE

RESULTS

3.1 Introduction

In Chapter 1, it was concluded that an opportunity existed to improve on current coronary stent coatings by the use of conducting polymers. The overall aim of current study was therefore to produce a series of conducting polymer coatings on stainless steel surfaces and the results of this work are reported in the present chapter. It was also highlighted in Chapter 1 that the electropolymerisation conditions used to produce conducting polymer coatings may affect the coating properties and drug release characteristics. Therefore, the effect of monomer and drug concentration on the coating properties and drug release profiles achieved will also be reported here. From this work, a set of coating parameters has been selected and used to produce an optimised coating on a stainless steel coronary stent. The drug release profile data generated from this optimised stent forms the conclusion of this chapter.

3.2 Effect of Pyrrole Concentration

3.2.1 Pyrrole concentration varied with 0.1 M Sodium Salicylate

The Pyrrole concentration within the coating solution may be an important factor in the quality of the Polypyrrole surface coating achieved by electropolymerisation. It was therefore decided to examine how varying concentrations of Pyrrole within the coating solution would affect the mass and integrity of coating produced, and ultimately its drug release characteristics. In the first set of experiments, Pyrrole concentrations of 0.1, 0.5, and 1.0 M in the presence of a constant Sodium Salicylate concentration of 0.1 M were examined.

- Analysis 1: comparing effect of varying Pyrrole concentration (0.1 M, 0.5 M, 1.0 M) on drug eluting coating properties when supported by 0.1 M Sodium Salicylate

	A	B	C
Pyrrole/Sodium Salicylate concentration (M)	0.1/0.1	0.5/0.1	1.0/0.1
Weight of coating (mg)	0.5 +/- 0.1	8.3 +/- 0.9	10.4 +/- 1.5
Coating Properties	thin, even, smooth	medium, even, rough	thick, uneven, rough
Initial (10 mins) Salicylate release (μg)	19.33 +/- 3.3	1362.06 +/- 200	1493.03 +/- 350
Total (approximately 28 days) Salicylate release (μg)	113.04 +/- 40	16 965.37 +/- 550	11 631.59 +/- 2000
Coating Integrity	no issues	consistent polymer debris	consistent polymer debris, polymer piece detachment

Table 3.1 Table of coating properties for stainless steel wires coated by potentiostatic electropolymerisation in a solution of 0.1 M Sodium Salicylate and 0.1 M, 0.5 M, and 1.0 M Pyrrole. All observations and data are based on an average of 3 wire repeats for each experimental condition.

The results in Table 3.1 show that by increasing the pyrrole concentration with a constant concentration of 0.1 M Sodium Salicylate in the solution produces films with larger weight. Similarly, by visual inspection it appears that the thickness of the coating increased with the increase in Pyrrole concentration. An increase in Pyrrole concentration produced an increase in the initial release of Salicylate (after 10 minutes of immersion). This increase was more marked when comparing 0.1 M Pyrrole wires to 0.5 M, and is less so when comparing 0.5 M Pyrrole to 1.0 M wires. The total Salicylate eluted over the full follow up period (30 days) demonstrate an increase from 0.1 M Pyrrole to 0.5 M, but this value actually decreases from 0.5 M Pyrrole to 1.0 M. The polymer coating also appears to become more unstable with the increase of Pyrrole concentration. The coatings produced with 0.1 M Pyrrole and 0.1 M Sodium Salicylate suffered from no observed degradation. However,

the 0.5 M Pyrrole was shown to be unstable, with observed polymer debris located in every physiological solution sample. This observation was more marked in the 1.0 M Pyrrole coatings, with the presence of more polymer debris throughout and actual degradation of significant amounts of the coating resulting in small pieces becoming detached from the main structure. These pieces of debris were clearly visible on visual inspection of the release medium solution.

3.2.2 Pyrrole concentration varied with 0.5 M Sodium Salicylate

In the second set of experiments, Pyrrole concentrations of 0.1, 0.5, and 1.0 M in the presence of a constant Sodium Salicylate concentration of 0.5 M were examined.

- Analysis 2: comparing effect of varying Pyrrole concentration (0.1 M, 0.5 M, 1.0 M) on drug eluting coating properties when supported by 0.5 M Sodium Salicylate

	D	**E**	**F**
Pyrrole/Sodium Salicylate concentration (M)	0.1/0.5	0.5/0.5	1.0/0.5
Weight of coating (mg)	0.7 +/- 0.1	24 +/- 1.5	80 +/- 6
Coating Properties	thin, even, smooth	medium to thick, even, rough	very thick, even, rough
Initial (10 mins) Salicylate release (μg)	111.37 +/- 4.5	1552.9 +/-190.7	2663.97 +/-350
Total (approximately 28 days) Salicylate release (μg)	318.1 +/- 150	11 763.98 +/- 725.1	10 276.4 +/- 1301
Coating Integrity	no issues	debris in first few days, nothing afterwards	fragile, polymer debris early and late, medium amount

Table 3.2 Table of coating properties for stainless steel wires coated by potentiostatic electropolymerisation in a solution of 0.5 M Sodium Salicylate and 0.1 M, 0.5 M, and 1.0 M Pyrrole. All observations and data are based on an average of 3 wire repeats for each experimental condition.

The results of weighing the produced coatings show that with increasing Pyrrole concentration in the coating solution, a greater weight of coatings are produced. The amount of weight gain between the 0.5 M Pyrrole and 1.0 M is very marked, and this is supported by the observed thick coating deposited on the stainless steel wire. In all the wires produced, from all the possible experimental condition ranges, the wire coatings produced by 1.0 M Pyrrole and 0.5 M Sodium Salicylate appear to be the thickest by visual inspection. The 0.1 M Pyrrole wires again showed a thin coating which was even in coverage and smooth in nature. The 0.5 M Pyrrole wires again appeared thick in nature, in a manner equivalent to that achieved by the wire B (0.5 M Pyrrole, 0.1 M Sodium Salicylate), however, the weight of 24 mg for the coating is larger than the 8.3 mg average achieved in B wires. All these coatings made from the varying Pyrrole concentrations (0.1 M, 0.5 M, 1.0 M) showed an increase in weight following the addition of 0.5 M Sodium Salicylate in place of 0.1 M Sodium Salicylate. The amount of Salicylate eluted initially (10 minutes) showed similar patterns to the Analysis Group 1 (Pyrrole varying with constant 0.1 M Sodium Salicylate) with the amount increasing with increase in Pyrrole concentration. Comparing the two groups also shows that the amount of salicylate eluted initially increases when the Salicylate concentration in the solution is increased from 0.1 M to 0.5 M for each Pyrrole concentration. The pattern of total Salicylate eluted matches that of the previous group, with an increase from 0.1 M Pyrrole to 0.5 M, but a drop off afterwards when increasing Pyrrole to 1.0 M. The values obtained for the total Salicylate release is higher when comparing the 0.1 M Pyrrole from the 0.1 M Sodium Salicylate group to that of the 0.5 M group, but those for the 0.5 M and 1.0 M Pyrrole wires are actually less in comparison. The coating integrity shows a similar trend to the previous group, with an increase in Pyrrole concentration resulting in increased fragility of the coating. At 0.1 M Pyrrole the coating suffers from no observed degradation or polymer 'dislocation'. The 0.5 M Pyrrole suffers from some observed polymer debris in the early immersion physiological solutions, but none after these. The 1.0 M Pyrrole group shows a fragility resulting in early and late immersion solution presence of polymer debris, but it should be noted that it is not to the consistent level of those in Analysis Group 1, particularly wires B and C. Generally, the coatings in this group show greater stability then their equivalent in the 0.1 M Sodium Salicylate supporting group.

3.2.3 Pyrrole concentration varied with 1.0 M Sodium Salicylate

In the third set of experiments, Pyrrole concentrations of 0.1, 0.5, and 1.0 M in the presence of a constant Sodium Salicylate concentration of 1.0 M were examined.

- Analysis 3: comparing effect of varying Pyrrole concentration (0.1 M, 0.5 M, 1.0 M) on drug eluting coating properties when supported by 1.0 M Sodium Salicylate

	G	H	I
Pyrrole/Sodium Salicylate concentration (M)	0.1/1.0	0.5/1.0	1.0/1.0
Weight of coating (mg)	0.2 +/- 0.05	14 +/- 0.6	108 +/- 9.5
Coating Properties	thin, even, smooth	medium, even, rough	thick, uneven, rough
Initial (10 mins) Salicylate release (µg)	1275.04 +/- 324	1760.3 +/- 285.4	239.93 +/- 5.5
Total (approximately 28 days) Salicylate release (µg)	1295.703 +/- 339	11 834.4 +/- 919	2044.2 +/- 20.7
Coating Integrity	no issues	no issues	very weak

Table 3.3 Table of coating properties for stainless steel wires coated by potentiostatic electropolymerisation in a solution of 1.0 M Sodium Salicylate and 0.1 M, 0.5 M, and 1.0 M Pyrrole. All observations and data are based on an average of 3 wire repeats for each experimental condition.

The wires produced under the increasing Pyrrole concentrations showed a greater gain in weight; through coating deposition. It is interesting to note however, that the picture of increased weight gain for all Pyrrole concentrations by increasing the Sodium Salicylate from 0.1 M to 0.5 M is not continued onto with 1.0 M Sodium Salicylate, except in the case of 1.0 M Pyrrole concentration. The

wires produced with a Pyrrole concentration of 0.1 M and 0.5 M show a decrease in the amount of weight gain when the Sodium Salicylate concentration is increased from 0.5 M to 1.0 M. Conversely, the coating for the 0.5 M Pyrrole wire with the 1.0 M Sodium Salicylate addition produces a coating of thickness less than that produced in the wires when supported by 0.1 and 0.5 M Sodium Salicylate. The 0.1 M Pyrrole coatings remain thin and smooth. The coating produced in conditions of 1.0 M Pyrrole with 1.0 M Sodium Salicylate are strange in the manner that they resemble that produced by 0.1 M Sodium Salicylate but with greater thickness, but do not share in the uniformity produced with 0.5 M Sodium Salicylate. The picture with the 1.0 M Pyrrole is further complicated when the initial Salicylate release is compared to those of lower Pyrrole concentration and to those of same Pyrrole concentration but reduced Sodium Salicylate concentration. In all cases, the initial Salicylate concentration noted is the smallest in all the comparisons mentioned. The 0.1 M and 0.5 M Pyrrole wires show the relative increase on the previous Sodium Salicylate level (of 0.5 M) for the initial Salicylate released. The exact same picture is shown for total Salicylate released as mentioned for initial release, with 1.0 M Pyrrole being less than the whole group of 1.0 M Pyrrole wires and less than 0.1 M and 0.5 M Pyrrole concentrations bound with a 1.0 M Sodium Salicylate concentration. The 0.1 M and 0.5 M Pyrrole showed an increase of total Salicylate release compared to the last group, with 0.5 M Pyrrole being more than 0.1 M Pyrrole for this group. Again though, the amount released by the 0.5 M Pyrrole group in this instance does not match that obtained with 0.5 M Pyrrole and 0.1 M Sodium Salicylate. The nature of the coatings for the 0.1 M Pyrrole and 0.5 M Pyrrole in this case show no real issues in terms of fragility or degradation. Again the 1.0 M Pyrrole with 1.0 M Sodium Salicylate goes against the foreseen trends, producing a coating that is very weak, producing evidence of polymer debris and contamination in all physiological solutions sampled.

3.3 Effect of Sodium Salicylate concentration

3.3.1 Sodium Salicylate varied with 0.1 M Pyrrole

The Salicylate concentration within the coating solution may be an important factor in the quality of the Polypyrrole surface coating achieved by electropolymerisation and effect the level of drug immersed into the surface. It was therefore decided to examine how varying concentrations of salicylate within the coated solution would effect the mass and integrity of the coating produced, and ultimately its drug release characteristics. In the first set of experiments, Sodium Salicylate concentrations of 0.1, 0.5, 1.0 M in the prescence of a constant Pyrrole concentration of 0.1 M were examined. The results of this are shown in figures 3.1, 3.2 and 3.3.

- Analysing 4: the effect of varying Sodium Salicylate concentration (0.1 M, 0.5 M, 1.0 M) on the drug release profile of produced polymer coatings when supported by 0.1 M Pyrrole

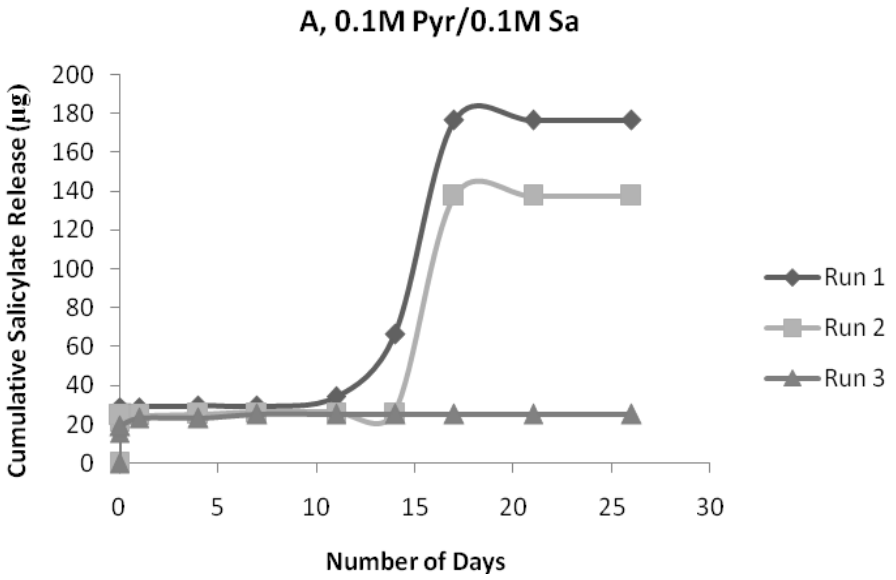

Figure 3.1 30 day cumulative drug release profile of 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of 0.1 M Pyrrole and 0.1 M Sodium Salicylate

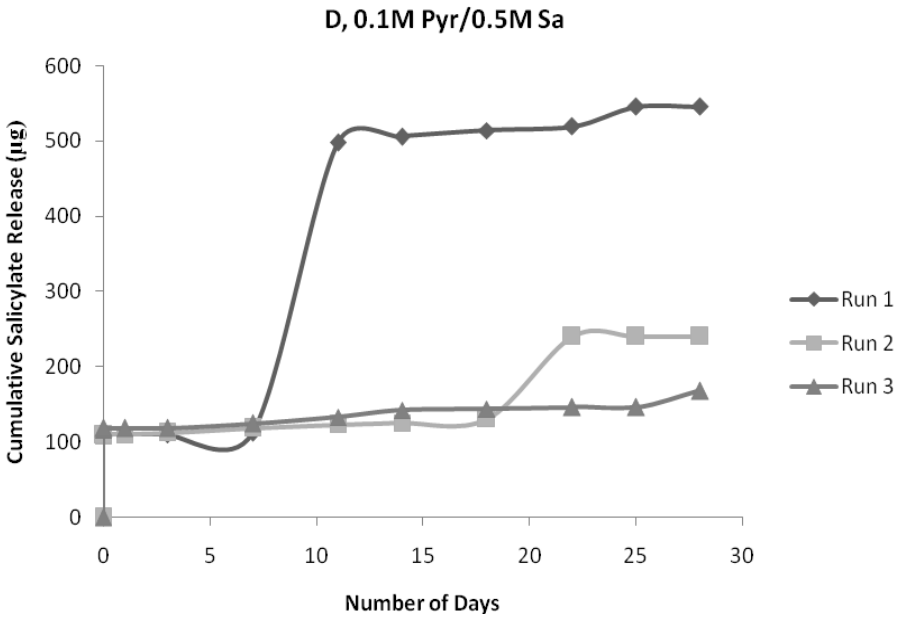

Figure 3.2 30 day cumulative drug release profile of 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of 0.1 M Pyrrole and 0.5 M Sodium Salicylate

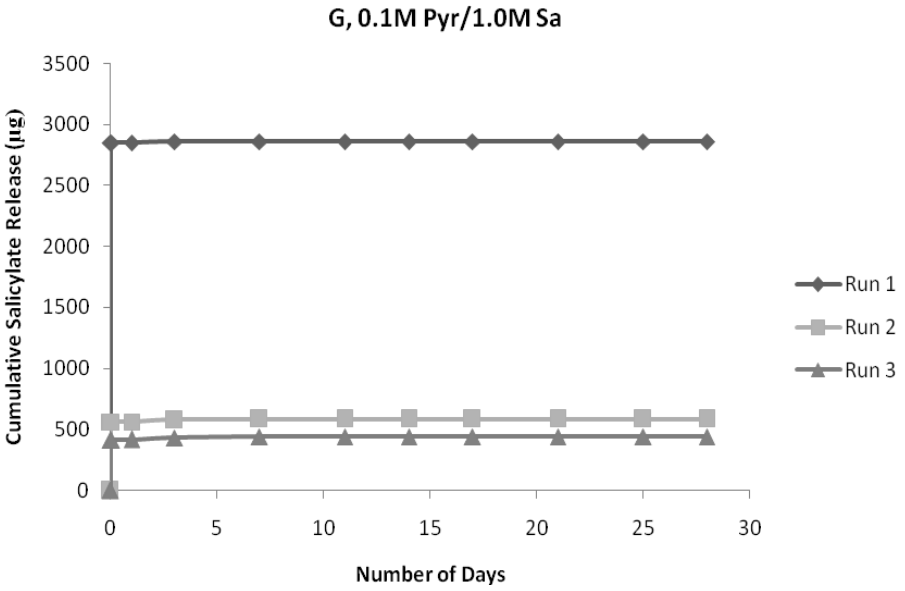

Figure 3.3 30 day cumulative drug release profile of 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of 0.1 M Pyrrole and 1.0 M Sodium Salicylate

The wires made of 0.1 M Pyrrole demonstrated that as the concentration of the Sodium Salicylate in the system was increased (0.1 M to 0.5 M to 1.0 M), the amount of Salicylate released on initial immersion (10 minutes) also increased. This same picture occured for the total release of Salicylate over the 30 day period, with increasing Sodium Salicylate concentration supporting the finding. The common picture of the drug release profiles for 0.1 M Pyrrole Concentration was the early burst release of most of the Salicylate; usually within the first hour, followed by a dramatic reduction in drug eluted over the following 30 days. However, the picture for 0.1 M Pyrrole wires with 0.1 M and 0.5 M Sodium Salicylate were slightly different in that they promoted a late burst (see Figure 3.1 and 3.2); usually at 11 to 17 days after the first immersion. This contributed up to 50% of the total drug release over the 30 day period. However, in the case of the 0.1 M Pyrrole wire with 1.0 M Sodium Salicylate (see Figure 3.3) and a number of the runs of the other 2 concentration conditions, 70% of the drug would be eluted over the first hour.

3.3.2 Sodium Salicylate varied with 0.5 M Pyrrole

In the second set of experiments, Sodium Salicylate concentrations of 0.1, 0.5, 1.0 M in the prescence of a constant Pyrrole concentration of 0.5 M were examined. The results of this are shown in figures 3.4, 3.5, 3.6 and 3.7.

- Analysis 5: the affect of varying Sodium Salicylate concentration (0.1 M, 0.5 M, 1.0 M) on the drug release profile of produced polymer coatings when supported by 0.5 M Pyrrole.

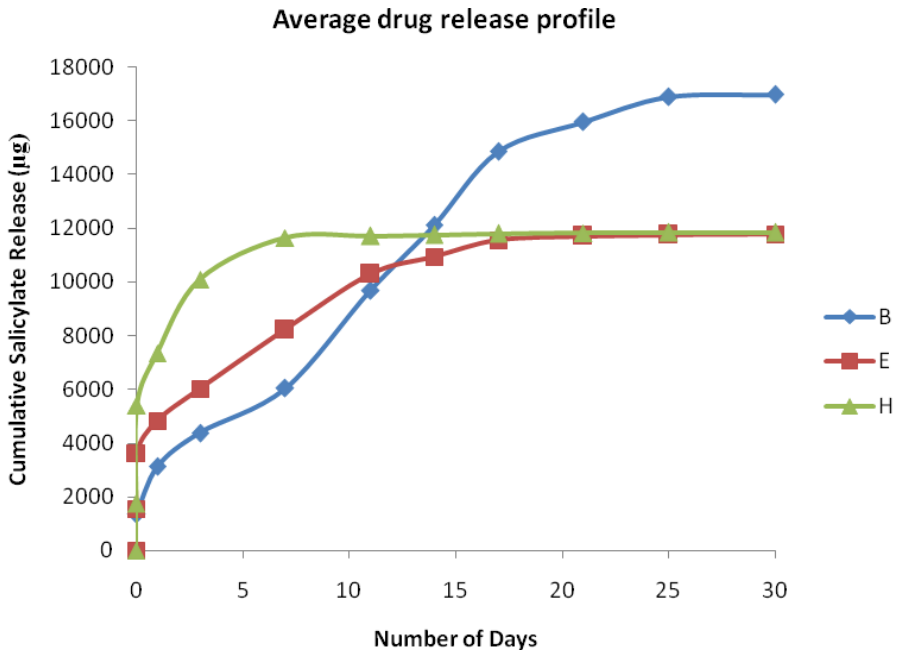

Figure 3.4 30 day cumulative drug release profile for the average from 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of B (0.5 M Pyrrole and 0.1 M Sodium Salicylate), E (0.5 M Pyrrole and 0.5 M Sodium Salicylate) and H (0.5 M Pyrrole and 1.0 M Sodium Salicylate)

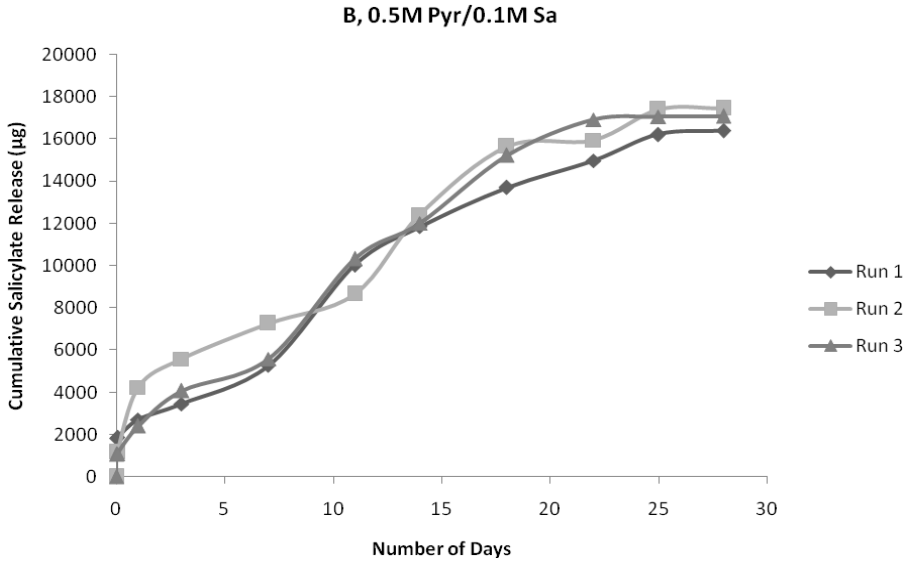

Figure 3.5 30 day cumulative drug release profile of 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of 0.5 M Pyrrole and 0.1 M Sodium Salicylate.

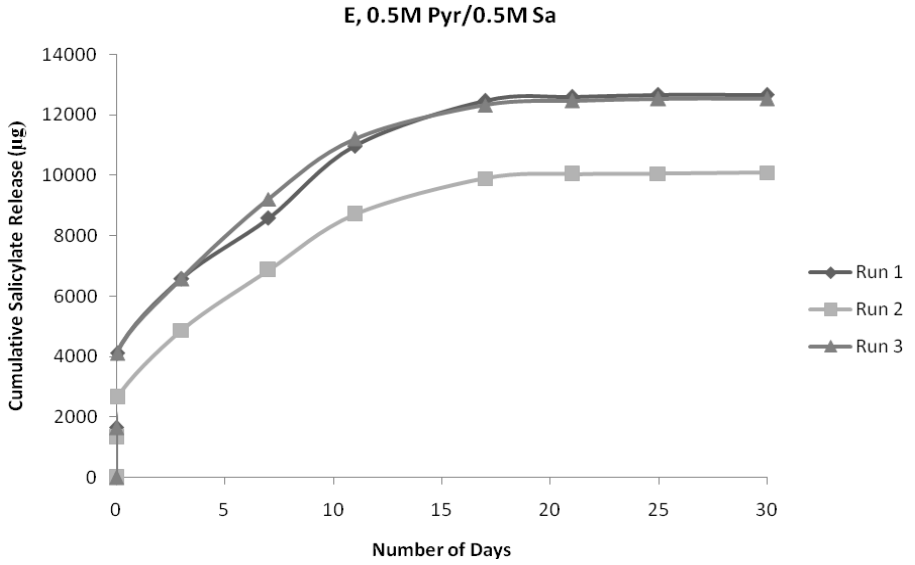

Figure 3.6 30 day cumulative drug release profile of 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of 0.5 M Pyrrole and 0.5 M Sodium Salicylate.

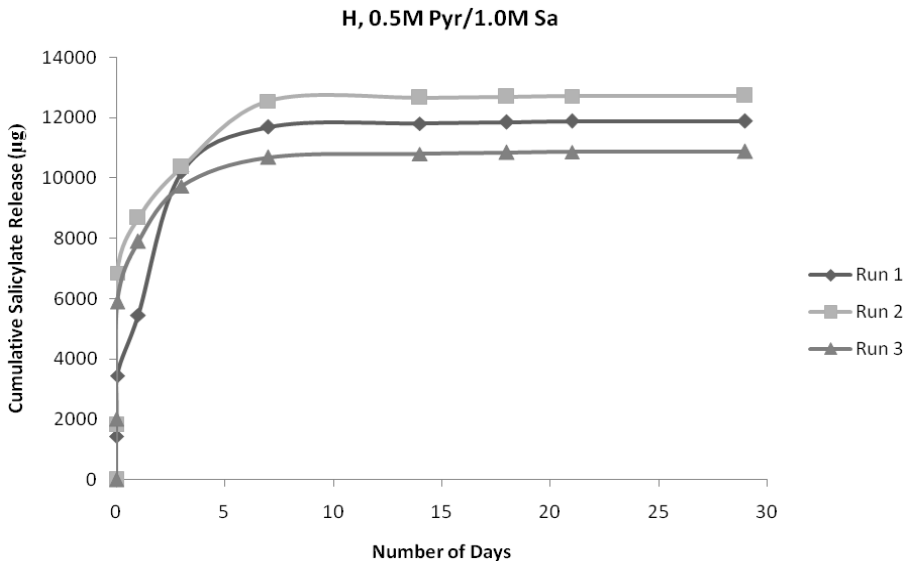

Figure 3.7 30 day cumulative drug release profile of 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of 0.5 M Pyrrole and 1.0 M Sodium Salicylate.

The wires produced using 0.5 M Pyrrole demonstrated that with increasing Sodium Salicylate concentration in the system (0.1 M, 0.5 M, 1.0 M) the amount of initial Salicylate eluted increased (see Figure 3.4). However, a similar picture was not demonstrated for the total Salicylate released over a 30 day period. The condition in which the highest total Salicylate was released was when the 0.5 M Pyrrole was doped with 0.1 M Sodium Salicylate. It is also interesting to add that there was only a small increase in total Salicylate eluted when comparing 1.0 M Sodium Salicylate doping to 0.5 M. The drug release profiles obtained in the 0.5 M Pyrrole coatings followed a trend of high and consistent Salicylate release till a certain point in the time period where the amount of drug being eluted begins to decrease (see Figure 3.4). By comparing the release profile, it was apparent that the point at which the amount of drug that was eluted began to drop differed depending on Sodium Salicylate doping concentrations used with the 0.5 M Pyrrole. Generally, as the concentration of Sodium Salicylate in the system was increased (0.1 M to 0.5 M to 1.0 M) the point at which the drug elution levels began to fall arrived earlier. In the case of 0.1 M Sodium Salicylate it occurs at around 22 to 25 days (see Figure 3.5), with 0.5 M Sodium Salicylate at 17 days (see Figure 3.6) and with 1.0 M Sodium Salicylate as early as 7 days (see Figure 3.7).

3.3.3 Sodium Salicylate varied with 1.0 M Pyrrole

In the third set of experiments, Sodium Salicylate concentrations of 0.1, 0.5, 1.0 M in the prescence of a constant Pyrrole concentration of 1.0 M were examined. The results of this are shown in figures 3.8, 3.9, 3.10 and 3.11.

- Analysis 6: the effect of varying Sodium Salicylate concentration (0.1M, 0.5M, 1.0M) on the drug release profile of produced polymer coatings when supported by 1.0M Pyrrole

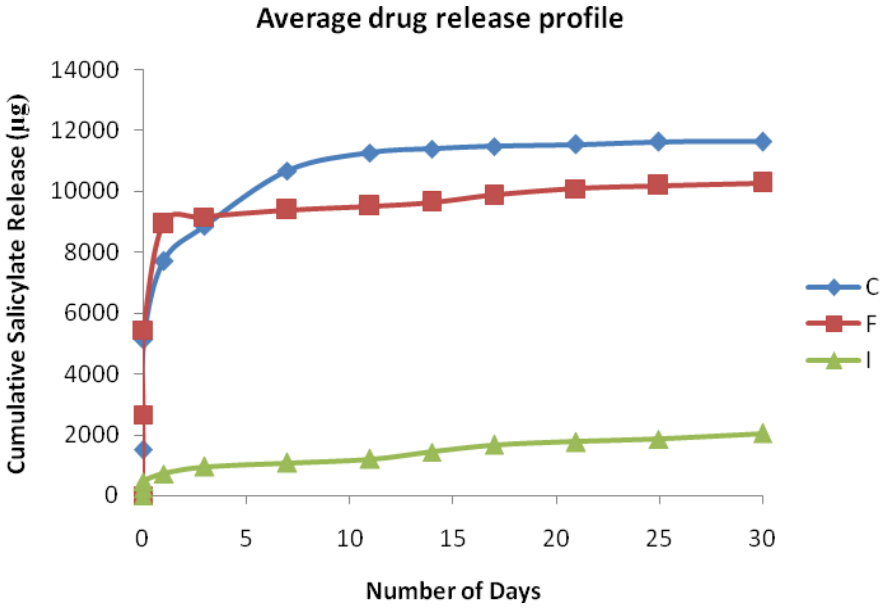

Figure 3.8 30 day cumulative drug release profile for the average from 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of C (1.0 M Pyrrole and 0.1 M Sodium Salicylate), F (1.0 M Pyrrole and 0.5 M Sodium Salicylate) and I (1.0 M Pyrrole and 1.0 M Sodium Salicylate)

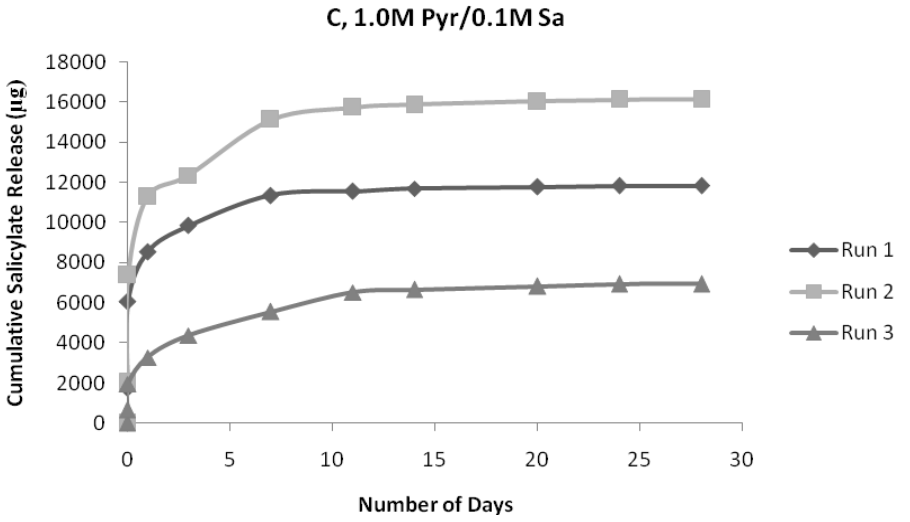

Figure 3.9 30 day cumulative drug release profile of 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of 1.0 M Pyrrole and 0.1 M Sodium Salicylate.

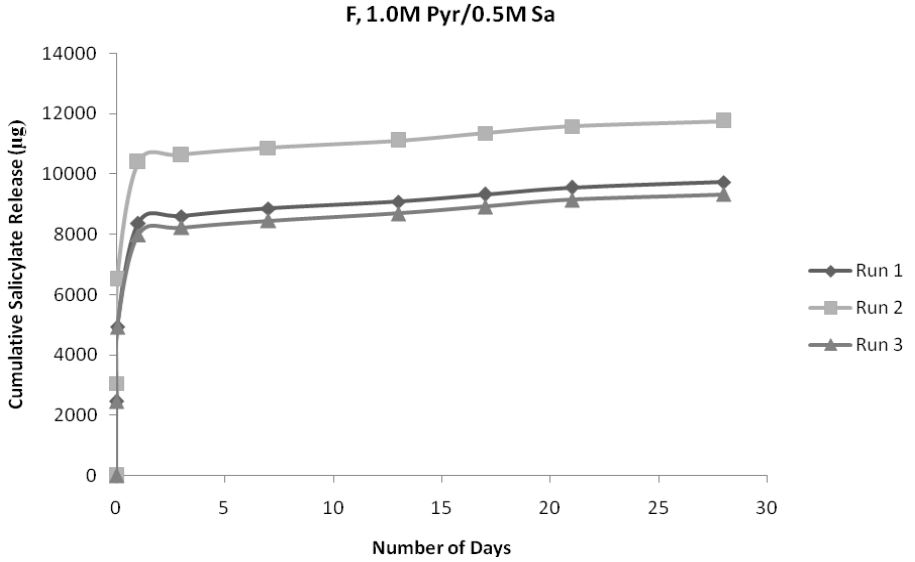

Figure 3.10 30 day cumulative drug release profile of 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of 1.0 M Pyrrole and 0.5 M Sodium Salicylate.

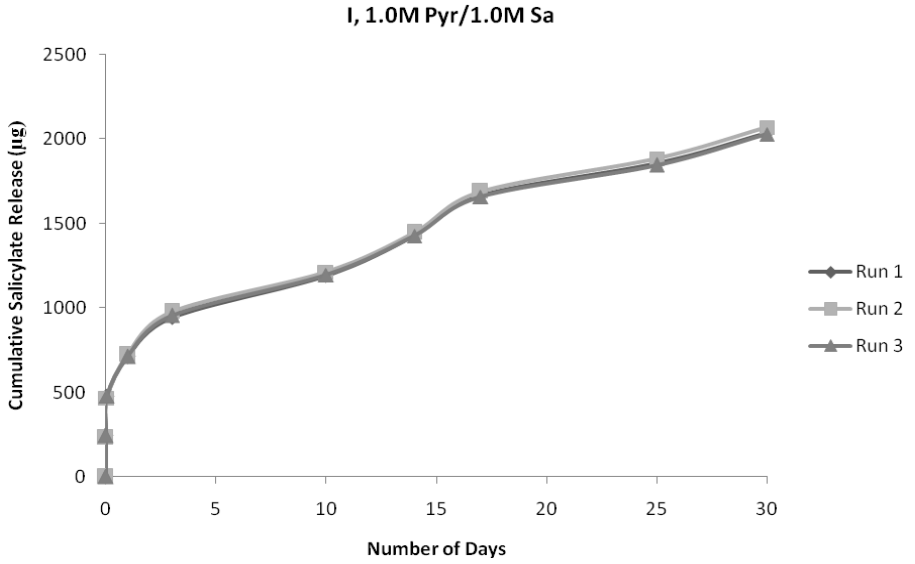

Figure 3.11 30 day cumulative drug release profile of 3 stainless steel wires produced by electropolymerisation (1.3 V, 10 min) in a solution of 1.0 M Pyrrole and 1.0 M Sodium Salicylate.

The release profiles obtained for the doping of 0.1 M Sodium Salicylate and 0.5 M Sodium Salicylate with 1.0 M Pyrrole followed the picture of early release with decreasing amounts after that, producing the characteristic negative exponential growth profile (see Figure 3.8). In the case of 0.1 M Sodium Salicylate concentration, the high levels of the drug were released over the first 7 days, and then the amount released steadily fell after that (see Figure 3.9). Whilst for the 0.5 M Sodium Salicylate wires, most of the drug was released over the first day, leaving a smaller consistent release over the remaining days of the 30 day period (see Figure 3.10). The release profile obtained for the wires made up of 1.0 M Pyrrole and 1.0 M Sodium Salicylate depict a picture not in keeping with any of the other profiles viewed before (see Figure 3.11). Initially a small Salicylate concentration is eluted (approximately 240 ug after 10 minutes) and following this, the amount eluted over the time periods remain in the narrow range of 230 to 240 ug until the last few days when the drug amount released drops. This is represented in the profile as a near linear increase of drug concentration with time from the 10 minute immersion point.

3.4 Coating of Coronary stent

In sections 3.2 – 3.3, the effect of Pyrrole and Sodium Salicylate concentration on the coating quality and drug release characteristics has been examined. From this work, proposed optimum coating conditions have been identified as, 0.5 M Pyrrole, 0.1 M Sodium Salicylate. In the present section, two standard bare metal stents were coated using these conditions and the drug release profiles recorded. The drug release from the first coated stent was examined with the stent remaining crimped onto the surface of the balloon. Following coating of the second stent, the balloon was inflated and the fully expanded stent removed for drug release measurement. Release profiles from each stent were then compared to a stainless steel wire coated under identical experimental conditions. The result of this is shown in Figure 3.12.

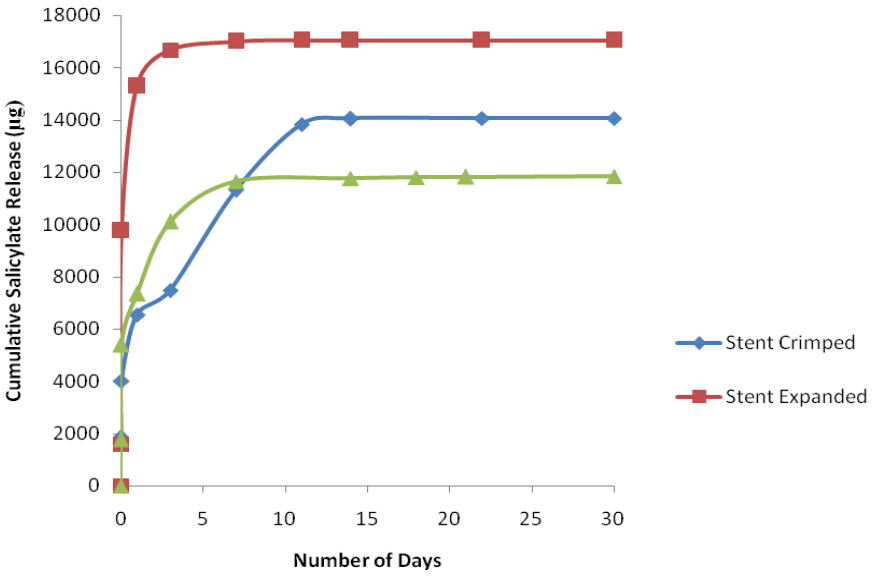

Figure 3.12 Cumulative drug release profile of Stent Crimped, Stent Expanded and Stainless Steel Wire (H, green line) in 0.5 M Pyrrole and 1.0 M Sodium Salicylate over a 30 day period

A formation of a coating on the crimped bare metal stents occurred, leaving a black residue film. However, following balloon expansion of one of the bare metal stents, a substantial part of the coating residue was lost with most of the remaining coating only covering the expanded metal struts. During immersion of the samples in the physiological solution over a 30 day period, the crimped bare metal stent and stainless steel wire showed no loss of polymer or observation of debris in the

solution. The expanded bare metal stent had regular positive observation of polymer debris in the solution sample up to 14 days following initial immersion.

The Salicylate release profiles for all 3 platforms are shown in Figure 3.12. All 3 coatings produced a similar initial Salicylate elution amount after immersion for 10 minutes. The data for the total elution of Salicylate over a 30 day period demonstrates that the greatest amount of drug was eluted from the expanded stent, followed by the crimped stent and then by the stainless steel wire. The release profile for the expanded stent shows us that there was a high early elution of Salicylate over the initial 3 day period, followed by a rapid decrease in elution after this until it reached no elution from 14 days onwards. The crimped stent showed a consistent high release of Salicylate up to 14 days, and then the release rapidly fell to nothing by the end of the 30 day period. The release profile for the stainless steel wire is high until the 7 day time point, after which it begins to consistently decrease.

CHAPTER FOUR

DISCUSSION

4.1 Introduction

Bare metal stenting became the treatment of choice for percutaneous coronary intervention, through its successful scaffolding of the diseased vessel and support against elastic recoil and abrupt closure. A recurring problem with its clinical use was the high rates of restenosis. As a result, development of stents led to drug-eluting types, which would release a drug in the vicinity of the diseased vessel to prevent the restenosis from developing. The two approved and most widely used drug-eluting stents were the Sirolimus and Paclitaxel eluting stents. Again, however, these stents were shown to suffer from the same problems of restenosis, albeit at a reduced rate, and they also suffered from other unforeseen issues as a result of their structure and mechanism of drug action. These include the delayed healing of the arterial endothelial layer due, in part, to the potent anti-proliferative effect of the drugs and a hypersensitivity response initiated by the polymer coating.

The proposition of conducting polymer technology as a coating for stents came about as a result of these negative findings and also as a natural response to the ever flourishing capabilities with conducting polymers. The hope is that conducting polymer technology may counteract the problems encountered in early generation drug-eluting stents, but also provide added beneficial aspects to the therapy, such as a biocompatible coating. Conducting polymer and electropolymerisation usage is based on an experimental understanding gained through work carried out over a number of decades. However, much of this work has focussed on the beneficial conductive properties. Only recently has interest been raised in the biocompatibility of these polymers and their potential to entrap biomolecules and provide targeted release of these molecules. Further to this, a limited number of studies have been performed to demonstrate and study the drug release properties of these polymer coatings, and even less information is available on the nature of release from a stent like structure.

The present study aimed to test the capacity for conducting polymers to coat a stainless steel metal structure, with an efficacious agent entrapped within the coating, allowing release of this agent over a sustained period of time. In addition, we aimed to observe the rate of release from a variety of coatings, and compare these to a desired profile of release. A focus was placed on observing the effect on the coating surface and release properties by a change in the balance of monomer and anion concentration used for electropolymerisation. The efficacy of coating a bare metal stent and supporting the release of the agent over time was also studied. The overall aim was to determine whether conducting polymers may act as a suitable platform for coating stents, with a future perspective on using this technology to produce drug-eluting stents for coronary heart disease patients.

4.2 Aims

4.2.1 Formation of coating

The results of this study have shown that our methodological approach was successful in supporting the formation of a conducting polymer coating on stainless steel wires and bare metal stents. This was evidenced by the visual formation of a coating of black residue (representative of Polypyrrole in the oxidised state) over the metallic surface, with a general covering of the surface and varying degrees of thickness in response to the particular parameters selected.

4.2.2 Salicylate binding

Evidence that Salicylate was incorporated into the polymer structure and supported the polymerisation process could be assumed initially from the successful formation of the film observed in our experiment. In addition to this, immersion of the coatings in a physiological solution and UV-vis spectrophotometric analysis of this solution, demonstrated the presence of Salicylate in the solution. The only means by which the Salicylate would have become located in the solution would be via its elution from the conducting polymer film. In a large number of films, this was not only demonstrated at the initial immersion of 10 minutes, but also at immersions throughout the 30 day time period. This allows us to assert that the presence of the Salicylate on the coating was not just as a result of attachment to the surface. It implies that the Salicylate may also have been entrapped in the inner layers of the polymer coating or chemically attached to the polymer chain. If the Salicylate was present only as a solution on the surface, then the period of 24 hours allowed for the wire to dry and the initial immersions would have removed this source from the surface. Hence, consistent release profiles over the 30 day period allow us to conclude that there must be additional mechanisms in play at supporting the elution. Our study has shown that Salicylate when in its dissociated form in solution, may act as an anion supporting conducting polymer formation. These findings were also made by Arbizzani et al in their use of Salicylate as a dopant (Arbizzani et al 2007). The results from our experimentation and work from previous studies will be able to shed more light on the mechanisms of elution and is covered in the following sections.

4.2.3 Drug release profile

The possible release of Salicylate (or other bio-active agents) is an important property of conducting polymers as drug-eluting stents. An additional important property is their potential to be able to control the release of such agents over a number of days. The current commercially available drug-eluting stents act to release most of the drug over a 30 day period, often with a negative exponential

decrease during this time (Grewe et al, 2000). This type of release profile supports the inhibition of the neointimal growth in the early stages whilst preventing excessive toxicity on cell growth in the later stages (Grewe et al, 2000). The intention of using conducting polymers as drug-eluting stent coatings would be to try and produce similar profiles, but also to examine whether the profiles may be further optimised and controlled by changing experimental electropolymerisation parameters. It may even be possible to create release profiles that reflect the mechanism of action of the selected drug, which may in some cases require a more sustained release rather than that necessary in the currently used antimitotic agents (Sirolimus and Paclitaxel). The results we have returned from most of our coatings demonstrate that the agent Salicylate, can be released in a controlled manner over the 30 day period. We have shown that the release rate and profile vary with differing experimental conditions applied. The relationship between the experimental conditions and the amount and rate of release will be discussed in section 4.3.3 and 4.4, with an attempt to establish some theoretical basis for the profiles of release developed. It is intended that this may in turn guide future development of conducting polymers, with a focus on selecting the most favourable conditions for a given application. Previous work on conducting polymers has highlighted a wide array of parameters known to affect the properties of the coatings produced (Guimard et al, 2007). Most previous research has mainly focussed on the effects on conductivity and mechanical properties of the film (Heeger, 2002). Our study set out to analyse the effect of changing the monomer concentration and anion concentration, used in the coating solution, on the produced release profile. Our results have clearly shown that differences in the drug release amount and rate can be produced by changing the experimental conditions of monomer and anion concentration. In addition, our results have also shown that certain relationships can be elucidated, describing how the changes in the concentrations and the ratio between the two may affect the release profiles.

4.2.4 Salicylate as an anion

As mentioned in section 3.1 our experimental method was successful in producing a polymer coating on the stainless steel wires and bare metal stents. The nature of the coating by visual appearance, thickness and weight were also shown to vary depending on the Pyrrole and Salicylate concentrations chosen. All experimental conditions applied were shown to support the formation of a coating. It may be proposed from this that both Pyrrole and Salicylate would be necessary for the formation of a conducting polymer film in this set up, with the Pyrrole providing the monomer units for the chain growth and the Salicylate acting as an anion supporting electropolymerisation. It has previously been proposed that only in the presence of an anion, an agent which provides the necessary electrons for the electrical balance of the chain, can the polymerisation process take place (Schuhmann, 2002). Our study has shown that Salicylate can act as this agent, and this is achieved by the negative ionic balance of Salicylate to the Sodium from which it becomes dissociated once in the

solution. It has been suggested previously that any agent that has a negative ionic balance and forms a solution in aqueous medium can act as an anion in the formation of a conducting polymer (Nalwa, 2007). This therefore opens up the possibility of entrapping an array of pharmacological agents within the conducting polymer coating, given that they could fulfil the aforementioned characteristics.

4.3 Pyrrole Concentration

4.3.1 Coating Properties

The different concentrations used for Pyrrole were all supportive in forming a coating film. 0.1 M of Pyrrole has often been used in previous studies (Arbizzani et al, 2007; Okner et al, 2009; Weiss et al, 2003) and again this concentration proved effective in producing coatings in the current study. In addition, increasing the Pyrrole concentrations to 0.5 M and 1.0 M still supported the formation of a coating. Our coating results show certain patterns on the coating properties achieved depending on the balance of Pyrrole and Salicylate concentrations utilised. The use of 0.1 M Pyrrole produced the thinnest coatings, with the smallest weight gain. This observation was consistent irrespective of any change in Salicylate concentration at this 0.1 M Pyrrole concentration. Pyrrole, being the monomer unit for Polypyrrole, is crucial for the formation of any coating on the metallic surface. At 0.1 M concentration it appears to produce films that seem to be small number of layers thick, with a weight gain in the region of 0.2-0.7 mg. The rate limiting step for polymerisation growth is described as the energy being available to form radical cations by oxidation (Genies et al, 1983). As polymerisation takes places, the concentration of Pyrrole in the solution available for forming radical cations will decrease. Therefore, as the concentration of Pyrrole decreases, less monomers are available to form polymeric bonds and hence, the rate of polymerisations slows. In our experiment the electrochemical reaction was run for 10 minutes. It may be proposed that this provided insufficient time for further chain growth in the 0.1 M Pyrrole experiment. Hence, a limited coating was formed. This reflects either a low concentration of Pyrrole to support the chain growth or a lack of time for the slower growth to progress. In either case, a low Pyrrole concentration is thought to be responsible for the results observed. Our findings have demonstrated that by increasing the Pyrrole concentration in the solution, a thicker coating can be produced. This may be related to the common mechanism of more monomers being available in the setting which supports a more likely chance of the radical intermediate cations being formed, which is necessary for the quicker chain length growth to proceed in the limited time period. Previous studies have not been able to quantify the upper limits on chain length but have proposed that as the energy in the outer radical cations falls, the chain length begins to stop increasing (Street et al, 1983). As our study used a constant time

frame, it is difficult to elucidate whether the increased thickness is as a result of longer chain length through greater energy potential or rather by virtue of a faster rate of chain formation in limited time. This is important to differentiate, because if the latter is the case, then it may be shown that with sufficient time the Pyrrole at lower concentrations may reach an equivalent thickness through repeated chain length production. This would be relevant in any commercial production of conducting polymers. The coating properties have also shown a change when the Pyrrole concentrations are increased from 0.5 M to 1.0 M. Generally the 1.0 M coatings appear to be more uneven, with gradients of thickness. The coatings made with 0.5 M Pyrrole appear to be more uniform in their thickness. This may be attributed to the occurrence of repeated chain growth length in the higher Pyrrole concentration through repeated monomer couplings preventing the chain from becoming unreactive or blocked. Hence, at the time the potential is ceased, a number of chains may be incomplete and hence, not covering the full length of the coating. Such an occurrence would produce the uneven surface topography obtained.

4.3.1.1 Salicylate changes

The 0.5 M Pyrrole wires and 1.0 M Pyrrole wires appear to show a variance in coating properties depending on what level of Salicylate is used to dope the monomer. These show that as the Salicylate concentration is increased from 0.1 M to 0.5 M, the thickness and weight of the coating will increase (see Table 3.1 and 3.2 in section 3.2 of results chapter). This further supports the view that the Salicylate provides the anions in the reaction, and hence, when increased promotes an increased rate of polymerisation. Previous studies have described how the anions work to stabilise the growth of the radical cations by supporting the coupling reaction over any secondary reactions (Otero & Santamaria, 1991). With greater Salicylate concentrations it may be understood that the coupling reactions and chain growth are further supported. However, at 1.0 M concentration of Salicylate there is a diminishing in the level of thickness of the coating and the weight gain achieved. It may therefore be proposed that the balance of the Salicylate at this concentration may be such that instead of supporting chain growth, the increased physical number of Salicylate molecules may hinder the necessary formation of radical cations by occupying more space in the solution, hence, affecting the rate of polymer formation.

It may be summarised from our findings that Pyrrole concentration is the most important determinant of coating mass. However, Salicylate provides a supportive role, which in the wrong balance may hinder the film formation. The coating thickness obtained is very relevant for the stent application, as a limit on the level of thickness is necessary to prevent excessive reduction in the luminal diameter of the treated vessel, but this needs to be balanced with a coating density that can provide sufficient drug release. The relationship between coating thickness and drug release will be discussed later in section 4.3.3.

4.3.2 Mechanical properties

An understanding of the mechanical properties of the coating provides an important insight into how these coatings may behave once inflated or inserted into a diseased vessel. Our analysis involved observing how the coatings responded to being immersed in a physiological solution. Our observations show that coatings formed with 1.0 M Pyrrole had the most fragile films. This was reflected in the loss of polymer coating during immersion. Most of the degree of loss involved the release of small particles of coating, which would build up at the bottom of the solution. However, in the case of the experimental condition 1.0 M Pyrrole to 0.1 M Salicylate, larger amounts of the coating were lost, with one of the wires suffering from a direct loss of around half of its coating. Building on what has been discussed with the 1.0 M Pyrrole experiments; the process of polymerisation appears to either go through a state of inefficient growth or incomplete polymerisation. The inefficient growth could be attributed to the increased monomer units altering the normal ratio of monomer to anion balance, favouring the monomer through a competitive pathway. This may result in more monomer units within the chain with respect to anions, reducing the electrical balance offered by the presence of the anions. Degradation may then be exacerbated by the immersion in physiological solution which would support the process of dedoping and ion for anion exchange. If this occurs with a loss of anions on an already electrically compromised chain structure, it may result in degradation at the points of anion loss, producing polymer debris that is larger in length because of the greater gaps between individual anions. The alternative explanation offered is that the uneven growth of the monomer chains results from incomplete polymerisation. As previously stated, chain length growth ends often at the stage of reduced reactivity in the end radical cation or when the ends become sterically blocked (Skotheim, 1986). Now in the case of excess Pyrrole and a 10 minute electrochemical reaction period, a number of polymerising chains may never reach such a state and hence, at the moment the coating procedure is ceased, the conducting polymer will remain attached to the metal and accompanying layers with part formed chain lengths. These part-formed chains would be susceptible to degradation following the immersion in the physiological solution. This explanation is supported by the progressive improvement of the coating's mechanical properties observed when the pyrrole concentration was decreased from 0.5 M to 0.1 M. It may seem that in the case of 0.5 M Pyrrole, the complications mentioned previously are present but to a lesser degree, resulting in reduced evidence of degradation. In the case of 0.1 M Pyrrole, no issues of degradation were highlighted. This is in keeping with the previous observations that 0.1 M Pyrrole provides complete polymerisation resulting in an even and strong coating.

The issues pertaining to coating thickness and mechanical properties are of relevance to our desire for developing a suitable drug-eluting stent. A control on coating thickness is necessary, to ensure

that it would not overwhelm the vessel's diameter, whilst avoiding fragile coatings, which if present may elicit emboli formation or inflammatory reactions via polymer debris. A number of publications have highlighted this issue with commercially available drug-eluting stents (Okner et al, 2009). They have observed the development of defects in the polymer coating following balloon expansion, resulting in potential risks of thrombosis, microemboli formation and inflammatory reactions due to dislodged polymer pieces. Achieving a sound coating in terms of mechanical strength, with malleability allowing elongation during expansion, with no risk of polymer degradation, must therefore be achieved whilst also delivering a suitable drug release profile. Hence, one of the aims of this study was to evaluate the effect of varying the concentration of coating solution parameters to be able to offer an optimised balance of concentrations for future application in stent coatings.

As before, it has been show that all wire coatings produced, as well as the stent coating, have shown evidence of Salicylate drug elution. The amount and rate by which the Salicylate is eluted varies depending on the experimental conditions (Pyrrole and Salicylate concentration) applied. This therefore provides the opportunity to explore relationships between variations in these conditions and possible release profile characteristics. The effects of varying Pyrrole concentrations, of varying Salicylate concentrations, and the balance between these will now be discussed with regards to the Salicylate release profiles achieved.

4.3.3 Drug release profile

Firstly, we will begin with reviewing the effect of varying the Pyrrole concentration on the coating's capacity to release Salicylate. Initially, the descriptions made of coating thickness and mechanical properties are relevant to this stage of the discussion. We've discussed how generally increasing the Pyrrole concentration has led to thicker/heavier coatings. However, at the higher ranges of Pyrrole concentration, the coating may be more fragile. A general assumption may be offered that with the thicker coatings we would expect a greater amount of Salicylate elution, given that the coating should be made up of 25-30% of the dopant Salicylate (Mui & Grunwald,1982). Each change in Pyrrole concentration (0.1M/0.5M/1.0M) was compared between themselves by contrasting their drug release profiles when supported by a constant Salicylate concentration. For 0.1 M, 0.5 M and 1.0 M Pyrrole with 0.1 M Salicylate it was shown that for increasing Pyrrole the initial Salicylate amount released (after 10 minutes) would increase. Knowing that the Salicylate concentration is kept constant in the three different Pyrrole concentrations, it may be proposed that the Salicylate that is bound at the surface accounts for the major component of release at the initial immersion. Hence, it may be presented that coatings that use the higher Pyrrole concentration usually involve the formation of a coating with a larger surface area, which can accommodate more of the Salicylate on the surface. Further, total Salicylate release over the 30 day period showed an increase from 0.1 M Pyrrole to 0.5 M Pyrrole concentrations, but a decrease when comparing the 0.5 M amounts to

that of 1.0 M (although these wires still eluted more than the 0.1 M wires). The increase from the 0.1 M Pyrrole wires to the 0.5 M may be attributed to the greater concentration of Salicylate found located within the conducting polymer coating. The mechanism of elution due to surface bound Salicylate has already been proposed in section 4.2.2. However, in the case of Salicylate being entrapped within the polymer layer or as part of the polymer chain, it can be offered that these processes would involve a longer time for elution to occur. The Salicylate entrapped in the layers would only be eluted following a process of diffusion (initial concentration gradient favouring outward diffusion) and this would take time for release, possibly reaching points of equilibrium in the immersion solution. The Salicylate found as part of the polymer chain may be eluted by dedoping or ionic exchange. In either case, this would be a gradual process, reflected in a release profile over the duration of 30 days. The large total release of the 0.5 M Pyrrole wire compared with 0.1 M wire, along with a release profile that shows consistent release rather than an early burst supports the presumption that the longer release is really occurring by the combined effects of diffusion, dedoping and ionic exchange. The wires produced with 1.0 M Pyrrole (and 0.1 M Salicylate) showed a reduced total elution as against the 0.5 M wires and had a profile picture which released until 14 days and then decreased substantially after this. A number of reasons are possible for why the 1.0 M Pyrrole coatings produced an inferior release profile, with either the low Salicylate (0.1 M) concentration used or the nature of coating's mechanical properties being contributory. In the first instance, such a high polymer concentration would require Salicylate to support the electrically neutral backbone necessary for polymerisation. Hence, a saturation point for polymerisation could be said to be reached, and any increase of polymer concentrations above 0.5 M would not benefit the polymerisation rate unless the anion concentration is increased. With this explanation it would be expected that the total elution rates should be equivalent to that of 0.5 M, with 1.0 M Pyrrole coatings demonstrating a saturation point of monomer to anion at that level. However, with a reduced elution amount, the second supportive reason for the decreased performance may be addressed, namely that the poorer mechanical properties correspond to reduced Salicylate elution. The mechanism by which this may occur is via the fragility and instability of the coating leading to debris formation and hence, the loss of Salicylate within these polymer clumps rather than into the physiological solutions. Our findings are supported by comparing coatings of 0.1 M to 0.5 M to 1.0 M Pyrrole made using a Salicylate concentration of 0.5 M. These include an increase in initial Salicylate release with increasing Pyrrole concentration and an increase in total drug eluted (except for the case of 1.0 M Pyrrole which has a little less total eluted amounts than the 0.5 M coatings). This similar pattern is also observed for the range of Pyrrole concentrations with an added Salicylate concentration of 1.0 M. It may be suggested that the benefits in increasing Pyrrole concentrations cease after a certain level, even when the reciprocal Salicylate concentrations are increased to balance it. The results may show that with an increased electropolymerisation time, it would allow the possibility of complete and desired polymerisation to take place with the higher Pyrrole

concentrations used. However, the size of potential coating thicknesses generated in such a condition would appear to be a difficulty. It should be noted that in wire coatings made with 0.5 M Pyrrole, increasing the Salicylate concentrations from 0.1 M to 0.5 M and 1.0 M produced an increase in the initial Salicylate released but a decrease in total Salicylate eluted for the latter 2 wires against 0.1 M Salicylate. The increased initial Salicylate released may be attributed to that being found on the surface. With the 0.5 M Pyrrole coatings, 2 obscure findings are presented: a decrease in the weight gain of the coating from 0.5 M to 1.0 M concentrations of Salicylate and a decrease in total eluted Salicylate despite a weight gain increase from the 0.1 M to 0.5 M Salicylate concentrations. The latter observation may be as a result of the increased Salicylate release via the many small particles being formed by the degradation in the immersion of 0.5 M Pyrrole (with 0.1 M Salicylate) in physiological solution. The mechanism of Salicylate elution may be different to previous examples of degradation, because the particles are smaller and more numerous in nature, providing a greater platform for Salicylate release rather than harbouring the agents within polymeric clumps. From 0.5 M Salicylate to 1.0 M Salicylate, the decrease in weight gain may point towards a point of saturation for the 0.5 M Pyrrole in forming polymerisation chains. Again, with saturation, an equivalent value to the saturation point would be expected (at least at those of 0.5 M) for the parameters, including the coating weight gain. This reduction could be accounted for by the increased Salicylate concentrations in the solution, hindering the mixing abilities of the Pyrrole monomers and the Salicylate with the electrode. Hence, it can be proposed that a Salicylate concentration up to 0.5M/0.6M Salicylate may provide sufficient anions for the Polypyrrole formation in the 10 minute time frame. The 0.1 M Pyrrole concentrations showed stable release profiles with changing Salicylate concentration. The initial and total release increased with increasing Salicylate concentrations. The release profiles also varied as a result of this, and this will be discussed later in section 4.4.1.1.

Comparing the effect of different concentrations of Pyrrole on the level of eluted Salicylate, it can be concluded that a change in Pyrrole concentration does affect the amount of Salicylate taken up by the polymer and the amount released. The relationship is generally linear with higher Pyrrole supporting higher Salicylate take up. However, an apparent saturation point is reached whereby the coating integrity suffers along with the amount of Salicylate that is available to be eluted. It may be presumed that at a rate of 0.5 M Pyrrole, the most suitable amount of polymerisation and Salicylate take up can occur. At this concentration, the most suitable Salicylate elution rates are achieved. However, with caution, the balance of Salicylate available for doping is important in achieving a desired uptake and release, with too little producing fragile coatings and too much affecting the polymerisation conditions and process. Hence, a balance must be sought between Pyrrole and Salicylate concentrations.

4.4 Salicylate concentration

4.4.1 Drug release profile

4.4.1.1 0.1 M Pyrrole

The role of Salicylate concentrations on the drug release amount and rate from produced coatings will now be further elucidated, with a focus on drug release profiles for these wires. The effect of varying Salicylate concentration on the 0.1 M Pyrrole group have been mentioned earlier, but a few more observations will be made with a focus on the drug release profile. As mentioned, with increasing Salicylate concentrations the wire coatings produce an increasing initial release of Salicylate and an increasing total release of Salicylate over the 30 day period. The graphs for release profile are very similar, with an early release of Salicylate in the first few days, followed by generally little or no more release after this. However, the 0.1 M and 0.5 M Salicylate wires show a characteristic late burst, were a large amount of Salicylate is eluted at later time periods of 10 to 15 days. This characteristic late burst is not found in the 1.0M Salicylate wires. The general method of elution from the wires can be presumed to be a combination of that occurring from the surface in the early stage and the later release (late burst) as elution from entrapped layers or anion release. This would explain how most of the Salicylate is eluted after the first few days. The late bursts may imply that Salicylate is available for elution but located further down in the layers, and only with time in immersion, can this source be released by diffusion. This may indicate a particular layering of the Polypyrrole chains, with the Salicylate in the solution being indirectly entrapped in the initial layers close to the metal electrode surface. It is possible that the 1.0 M Salicylate did not have this extra entrapment because of the increased and immediate rate of coating formation over the wire surface. The findings with the 0.1 M Pyrrole concentration wires indicate that a coating can be formed that would be stable over an immersion period of 30 days. The coating has the potential to release Salicylate, however, its release is immediate in nature with the potential for late bursts which do not match the desired rate of early release with a slow exponential decrease over the 30 days.

4.4.1.2 0.5 M Pyrrole

In the case of 0.5 M Pyrrole wires, varying the Salicylate concentration produced mixed results. Generally, the initial release (after 10 minutes) increased with increased Salicylate concentration used. This points towards a surface based release of the drug. However, the total release achieved by the lower Salicylate concentration group is higher than the other two groups (0.5 M and 1.0 M Salicylate). The drug release profile of all the 0.5 M Pyrrole wires could be described as a consistent release of Salicylate for a number of days before a fall in the eluting amounts. A somewhat decreasing exponential release of drug is then observed to occur. Firstly, taking the coating produced

by conditions 0.5 M Pyrrole and 0.1 M Salicylate, it shows a consistently high release of Salicylate (in the range of 1500-3500 µg) up to around 25 days and then this release amount begins to decrease. Generally it has been stated that drug-eluting stents achieve drug elution dosages of 135 µg and 85 µg per stent for Sirolimus and Paclitaxel stents respectively (Geetha et al, 2006). The higher numbers achieved in our study are as a result of a longer length coating with greater thickness, hence, greater volumetric coatings are being produced. Given that production of a thick film coating negatively effected our expanded bare metal stent trial (see section 4.5), it can be inferred that any desired coating would require a strength which would allow suitable expansion of the coating by balloon inflation and alongside this, avoid a coating thickness that may impede blood flow. The release profile achieved for 0.5 M Pyrrole with 0.1 M Salicylate may be undesirable as it provides a consistently high release of the drug over the 30 days. The drug-eluting stents of Sirolimus and Paclitaxel aim for a two phase approach with an early rapid burst release followed by a slow release phase (Venkatraman & Boey, 2007). The early rapid burst would account for a total release of 50-60% of the drug dosage over the first week followed by a 20% further release up to 21 days and reduced amounts up to 30 days post implantation. This is believed to provide inhibition of neointimal growth whilst allowing reendothelialisation of the vessel and stent surface to take place, a process known to be necessary in promoting healing in the vessel intima and reducing the risk of late thrombosis. The profile of the 0.5 M Pyrrole with 0.5 M Salicylate coating combination shows a consistently high release of drug for up to 17 days. This may be described as somewhat of a reciprocal to an early rapid burst period, however, often the drug concentrations released amounted to 300 – 1200 µg per day. Again for drug-eluting stent applications these may be deemed as high Salicylate release amounts. However, the tumour suppressant agents used in commercial drug-eluting stents may require a smaller dosage then the anti-inflammatory agents we have chosen. Tumour suppressant agents such as Rapamycin (Sirolimus derivative) are highly hydrophobic, which implies that they are readily retained in the arterial walls. In contrast, salicylate is hydrophilic in nature and hence, would be rapidly washed out from the arteries. Therefore, a larger dosage of the hydrophilic agent (salicylate) would be required compared to a hydrophobic drug like Rapamycin to provide an equivalent therapeutic concentration. The nature of the release profile necessary may also be different, with acute and chronic phases of the inflammatory responses being targeted. Generally, the inflammatory cell responses persist over the 30 day period before being replaced by long term smooth muscle cell proliferation and migration (Grewe et al, 2000). Therefore, Salicylate's mode of action may be more suited to a consistent release over a 30 day period. This supports the profiles obtained for 0.5 M Pyrrole with 0.1 M and 0.5 M Salicylate. It is interesting in comparing these two release profiles with the final one of 0.5 M Pyrrole with 1.0 M Salicylate (see Figure 3.4). In this release profile the initial high burst lasts for up to 7 days before a consistent and rapid decrease in Salicylate release until the 30 day mark. The average release amount in the early burst could be estimated as 1000 µg per day in the first 5 days. In comparing the 3 release profiles of coatings

produced with 0.5 M Pyrrole but varying concentrations of Salicylate, it is apparent that the position at which the early burst stage ceases and the slow release stage begins becomes earlier as the Salicylate concentration is increased in the system. This pattern may support the assumption that most of the Salicylate, in its higher concentrations for production, is located on the surface layers rather then deeply entrapped or penetrated. This analysis is apparently contrary to the expected effects, with higher levels supposedly supporting polymerisation rate. At the higher concentrations of 1.0 M Salicylate we have found reduced polymerisation capacity and Salicylate elution, thought to be a result of saturation of the Salicylate within the solution, hindering the electrochemical concentration gradients around the electrode and actually disturbing the polymerisation process. One of the major supportive factors for the changing release profiles may also be that greater Salicylate release occurs in the coatings with less Salicylate concentration due to a greater degradation capacity. This provides a mechanism which supports the prolonged consistent release of Salicylate. Hence, a greater amount of Salicylate is eluted from the wire from 0.5 M Pyrrole and 0.1 M Salicylate compared to the same coating at 0.5M Salicylate. This is supported by work looking at Paclitaxel release by direct elution or support with a primer base coating or an over coating, which promotes slower diffusion based release. The fragile single polymer layer, offering direct elution, seemed to release more drug as a result of easier degradation of the polymer layer compared to the other coating types (Okner et al, 2006).

4.4.1.3 1.0 M Pyrrole

It is clear by observing the mechanical properties of the coatings produced by a 1.0 M Pyrrole concentration that they are mostly fragile. In all three Salicylate concentration levels examined, there is a certain level of fragility, which was demonstrated by the regular observation of polymer debris deposited within the immersion solutions. As has been previously proposed, this may be that a high monomer concentration may support the extended chain growth in the time period without any completion of the chain length. This implies uncompleted polymer chains at the end of the process, resulting in a coating more susceptible to degradation. The release profile for the 1.0 M Pyrrole wires appear to be similar for those at 0.1 M and 0.5 M Salicylate preparations, in which, a high release of Salicylate occurs over a number of days before the elution amount begins to reduce for the rest of the time period. Comparing the two release profiles, it is observed that the profile with less Salicylate added in the solution shows a high release profile up to 7 days followed by a sharp fall in drug release. This may be indicative of drug elution occurring via surface bonds in the early period followed by a supposed release from entrapped layers. This amount appears to fall off as a result of the loss of polymer coating with immersion and degradation, and hence, removing large proportions of film available to elute the drug. With 0.5 M Salicylate concentration experiment, it appears that the initial burst (attributed to surface bonds and outer layer elution) of Salicylate

occurs over a much shorter initial period. This follows a similar profile to that shown for the 0.5 M Pyrrole wires, with a key finding that Salicylate concentrations are necessary in large enough amounts to support the polymerisation process, and to protect against fragility, but not too high that they negatively affect the polymerisation rate and ratio; and hence produce a release profile showing quick early elution of the drug. A key aspect with the drug release profiles for 0.5 M and 1.0 M Salicylate supporting the 1.0 M Pyrrole is the consistent release of small amounts of drug during the time period. This was normally in the range of 230 to 240 µg and may be indicative of the higher Salicylate concentration producing more evenly entrapped drug via incorporation into the polymer chain.

4.5 Coronary stent coating

Our investigation is one of the few studies that has aimed to coat coronary stents with a conducting polymer and analyse its drug releasing potential. Previous work in this field has demonstrated the potential of such an approach (Weiss et al, 2003; Arbizzani et al, 2007; Okner et al, 2009) and our work has advanced this field by examination of different drug and monomer concentrations. We therefore wanted to determine if the results obtained in stainless steel wires could be extended to commercially available bare metal stents. The use of a bare metal stent as the working electrode was presumed feasible, given that the metal stent is stainless steel in type. Our experimental work was able to demonstrate the ability of producing a conducting polymer coating over the bare metal stent. The exact nature of this coating – specifically focussing on thickness, structural characteristics and mechanical properties would be dependent on the interplay of the bare metal stent as an electrode to support oxidation and polymerisation, along with the experimental parameters selected in the electropolymerisation process. By comparing the properties of the coating and drug release profile to that formed on a stainless steel wire (under the same experimental parameters), we were in a better position to appreciate possible effects of the bare metal stent material and geometry on coating formation and function. As described, we were successful in forming coatings on the bare metal stents. The coatings were relatively thick, matching the morphology of the coating formed on the stainless steel wire. The real difficulties with the selection of experimental conditions for this coating became established when one of the stents was expanded by balloon inflation. The result of this expansion was a loss of a substantial portion of the polymer coating. Rather, then the loss of sections, any polymer film that was not in direct coverage of the stent struts were displaced following expansion. This resulted in an immediate loss of polymer coating from the bare metal stent, which could have feasibly harboured parts of the Salicylate drug to be eluted. The effect of this destruction of the coating on the complete stability of the coating was unknown. The key issue which may be raised from this response to expansion is that such a response is not only undesirable in

clinical application of stents but potentially fatal. The loss of parts of the polymer had released many mini fragments from the polymer film. If this occurs in any of the vessels of the body's circulatory system, it could elicit the formation of microemboli with potentially fatal effects via obstruction of circulatory vessels. The remaining coating was generally well covered over the stent struts, with small areas of non-coverage – which may have been as a result of loss following polymer breakaway after expansion. Following the immersions of the coatings in physiological solution for a 30 day period, regular evidence was observed of polymer debris formation within the solution of the samples containing the expanded stent. This observation was consistent throughout the immersion period, except for the final few immersion samples (after 22 days). Again, this implies that the formed coating was weak initially, and hence, susceptible to degradation, or may be reflective of the coating's susceptibility to breakdown following pronounced immersion in physiological solution. Both cases present a scenario that is very much undesired in drug coating technology because of the detrimental effects that such breakdown would have on the coating's properties and the risk of micoemboli formation and induced inflammatory reactions from the degraded debris. The crimped stent showed no signs of degradation whilst kept in the immersion solution; this matched the picture with the stainless steel wire. This may highlight that the nature of the stent's metallic property is not the cause for the debris loss in the case of the expanded stent, but rather the nature of the geometric array of stent struts along with the initial damage to the coating structure, along with an increased surface area of exposure to the solution, producing the observations noted. The initial amount of Salicylate released by the expanded stent, crimped stent and stainless steel stent were similar. However, it should be noted that initially the expanded stent eluted less Salicylate than the other two coatings. This may be surprising because it is presumed that the increased surface area offered by the stent geometry should favour elution from the expanded stent via a surface bound means or diffusion from internal layers. It may be offered that the loss of polymer caused by the initial expansion may have reduced the amount of polymer on the bare metal stent and that more polymer was present on the crimped and stainless steel wire in bulk. The crimped stent produced a higher level of initial Salicylate elution then the stainless steel wire. This is interesting because the length and cross sectional area of the coating is larger for the stainless steel wire when compared to the crimped stent. This could therefore possibly point towards a stronger capacity for the bare metal stent coatings to elute Salicylate when compared to stainless steel metal wire coatings. In terms of total Salicylate eluted from the coatings over a 30 day period, the expanded stent showed the highest levels. This may be surprising given the loss of polymer on expansion, the thinner coatings remaining on the stent struts and the bare patches on the stent struts providing a supposed reduced platform from which the Salicylate may be eluted. This large elution may have been as a result of the multiple polymer pieces that degraded from the coating over the first two-thirds of the immersion period. Alternatively, the stent strut geometry may support the diffusion based Salicylate release from these thinner layer coating. The release of salicylate observed for the expanded stent provides

a profile of high early release which is often indicative of surface bound elution. However, the initial picture may represent the rapid diffusion of the Salicylate from the stent strut coatings, which appeared less thick than that on the crimped stent and the stainless steel wire. All Salicylate was eluted by 14 days, demonstrating a complete elution of either the surface bound or entrapped drug agent. The crimped stent produced a higher total elution of Salicylate over the 30 days then the stainless steel wire coating. This again may offer support to the view that the bare metal structure may provide a more conducive platform for the bound film to elute the drug. The release profile also produces a high release up to 14 days before a rapid decline, in contrasts to the limit of 7 days in the stainless steel coating.

It should be noted therefore, that bare metal stents can be coated with a conducting polymer by the electropolymerisation method. The stent structure may appear to be more supportive to the release of Salicylate agent then the stainless steel wires. We were also able to show that a precoated stent can still elute the Salicylate agent over an approximately 14 day time period, following balloon expansion. Some issues of caution involved the concern with polymer loss on expansion and polymer degradation during the immersion in a physiological ionic solution. In addition, we have demonstrated that it is feasible to coat the outer portion of the bare metal stent and still allow expansion with certain properties of drug elution still intact.

4.6 Summary of Findings

Our study has allowed us to obtain a greater understanding of how two key parameters, monomer concentration and anion concentration, may impact on a number of properties of conducting polymers as drug-eluting stent coatings. These properties include the coating thickness, the coating's mechanical properties and the coating's drug release profile. The findings highlight the importance of achieving the balance between the parameters of Pyrrole concentration and Salicylate concentration used in the electropolymerisation method. Some of the clear links and findings from our study can firstly be presented. The coatings formed by using 0.1 M Pyrrole produced thin coatings that were generally stable during their immersion in physiological solution. The problem with viability of 0.1 M Pyrrole coatings for drug-eluting stent applications seem to be the low amount of drug eluted and drug release profile which only acts over a very short period of a few days. It was generally demonstrated that the coating thickness may be increased by raising the Pyrrole concentration in the system. Separately, the coating thickness may also be increased by raising the Salicylate concentration within the system. In both cases, there were exceptions and there is evidence that a balance of the two parameters work together in determining not only the coating thickness but also its properties of stability and drug eluting capacity. Overall, the Pyrrole

concentration was found to be the most important determinant of coating thickness. In contrast to the findings with 0.1 M Pyrrole, we discovered that using 1.0 M Pyrrole concentrations led to the production of fragile coatings. Generally, increasing the Salicylate concentration in the system could make the coatings more stable. However, in the case of 1.0 M Pyrrole, this was still not sufficient in preventing the issues of instability in the coating. Again, it was found that by increasing the Pyrrole concentration, the amount of initial drug eluted from the coating was increased. However, for the highest total Salicylate elution over the 30 day time period, coatings produced with 0.5 M Pyrrole produced the best results. The strongest drug profile obtained was by the conditions 0.5 M Pyrrole with 0.1 M Salicylate. The problem with this coating however, was that the film produced would be too thick for commercial use on a bare metal stent. This was highlighted by our coating of a bare metal stent with the conditions 0.5 M Pyrrole and 1.0 M Salicylate, which produced a coating that suffered from a substantial loss of polymer on balloon expansion. Hence, it may be ascertained that despite its strong drug release profile, its use in actual practice would be limited by its thickness. Secondly, the coating produced under these conditions was shown to be very fragile. This component would also make any potential application unfeasible. The wire made from 0.5 M Pyrrole and 0.5 M Salicylate is another good example of a desired drug release amount and profile. Compared to 0.5 M Pyrrole/0.1 M Salicylate, it provides a greater level of stability. However, its level of thickness would again make it impractical in the application setting. The final wire made using 0.5 M Pyrrole involved the use of 1.0 M Salicylate. These experimental conditions were used to coat a stainless steel wire and bare metal stent. Success was had in coating these devices, with the coating's mechanical property being stable, except in the case of the expanded stent. Due to the initial thickness of the coating, the balloon expansion involved the loss of polymer on inflation. Following on from this, there was consistent evidence of polymer debris in the immersion solutions. These experimental conditions were however successful in providing a drug release profile for the stainless steel wire, crimped stent and also the balloon expanded stent over a 30 day period. Any future development of this technology would require reducing the thickness of the coating. The best strategy for this would be seeking an optimum Pyrrole concentration between 0.1 and 0.5 M.

4.7 Future Perspectives

4.7.1 Design requirements

A key aspect of any future use of conducting polymer method was the application of these coatings to the bare metal stents. Firstly, given the size of bare metal stents, a very thin coating would be required so as not to hinder the percutaneous delivery of the stent and prevent any hindrance on its haemodynamic and general geometric profile. Secondly, a coating that has a certain level of

malleability would be required, to support the expansion of the coating on balloon inflation. It has been demonstrated that under certain electropolymerisation conditions, conducting film polymers of the order of 20nM can be produced (Guimard et al, 2007). Our own study has shown the development of very thin coatings which would be stable on the stainless steel wire and during immersion. Unfortunately, the study time never allowed the coating of a bare metal stent with these coating requirements (0.1 M Pyrrole) and the consequent expansion by balloon. However, work in our laboratory has involved developing conducting polymer coatings of the same given thickness which has shown success in coating stents, allowing undisturbed expansion with these specifications.

The optimum designs for any clinical application of the conducting polymer technology entails that coatings can be produced on the bare metal stents without negatively affecting its properties through the process. The eventual coating should form a thin layer over the inner diameter (or outer if it is desirable for drug to be eluted into the lumen), with a coverage over the metal struts only. The coating must be able to expand in relation to the expansion of the stent and not lose its mechanical properties whilst doing so (this includes surface cracking). It is in this setting, in the patient's diseased vessel, that the drug should be eluted from the stent over a period of pre desired time. Key properties include that the coating structure does not degrade through the force of the flow of blood, or as a result of the ionic or pH environment. This is to maintain the perceived biocompatible coating support over the stent and also to avoid the development of microemboli from any polymer debris. The long term efficacy of the coating's mechanical property is therefore also stressed. Our study adds to the current knowledge base on conducting polymers as drug-eluting stents (Weiss et al, 2003; Arbizzani et al, 2007; Okner et al, 2009), although it is clear that much additional research needs to take place before a conducting polymer could be used clinically. However, the present study has provided some preliminary evidence to indicate that the desired thin coating, with malleability, biocompatibility, structural integrity and ability to elute a drug at a desired rate and amount are all different properties that can be potentially achieved by varying the parameters in the electrochemical process.

4.7.2 Future Study

Future studies should act to observe the effects of other synthesis parameters on the polymerisation formation of the coating. A number of interesting parameters include the acidic nature of the solvent, temperature of synthesis and water content of the solvent. These parameters have previously been shown to affect experimental responses such as polymerisation reaction rate and polymer chain length, resulting in marked changes to coating structure and mechanical status (Downward & Pletcher, 1986; Rodriguez et al, 1987; Nalwa, 2007). Our own study involved two clear unknowns, the solvent acidic level and the level of dilution in each solvent mix. Using Salicylate as a dopant within the solution already favours an acidic condition, which has been shown to support the

polymer reaction (Nalwa, 2007). Equally, by varying the levels of Pyrrole and Salicylate in the aqueous medium, we were affecting the water content ratio of the final solution. In our setting it was difficult to keep such variables constant, however, an appreciation of their effect on the coating formation and hence, its subsequent drug release profile, would be informative. Any future study replicating our methodology may act to analyse acid and water status of the solvent and attempt to derive patterns describing how these variables impact final products. It is this array of possible experimental parameters, along with the substantive effects on coating properties that make conducting polymer technology use so exciting. Only with more experimental data, could a greater understanding of the proper specifications of generated coatings be developed. Much more is still unknown about the direct and indirect affect of these parameters on the production and hence, the best specification for use in drug-eluting stents.

4.7.3 Future Applications

Our understanding of electropolymerisation provides us with assurance that the Salicylate acts as a dopant agent in our experimental method. Previous work in the laboratory, along with previous studies using Salicylate as a dopant ion, and in particular the work by Arbizzani et al, 2007, has highlighted Salicylate's potential. Our study has shown success in using Salicylate as a dopant ion and the success in using the doping procedure as a method of optimising drug delivery and release. This opens up the possibilities for the use of conducting polymers as a drug-eluting stent using a whole host of pharmacological agents to reduce thrombotic risks related to stent insertion. The versatility of the properties available in the production of conducting polymers make it feasible for a number of agents to be entrapped or doped at once, along with control over the drug release routine of these multiple agents. Further work in conducting polymers cannot only open up the technology for coronary artery stents but a whole host of drug delivery and tissue engineering applications. Namely the capacity to functionalise the coating by varying the side chain bonds or forming covalent bonds with the monomer units allow a surface to be produced which could be selective for cell adhesion. In a coronary artery stent setting, this could favour the attachment of endothelial cells whilst inhibiting the attachment of unwanted cells such as platelet adsorption or smooth muscle cells.

4.8 Limitations

A number of limitations need to be addressed, which may have potentially affected any of our final results and conclusions. A key aspect of our methodological approach was the estimation of drug elution amounts over a 30 day period by immersing the conducting polymer coating in physiological solution over the 30 day period. However, the physiological solution the coating was immersed in

was not kept constant but rather changed at every time point. Hence, the data would show how much Salicylate was eluted between 10 minutes to 1 hour after immersion, and how much was eluted between 14 to 17 days after immersion, for example. Hence, it is argued that this provides a track of the amount of elution of Salicylate over the period of time. However, it may be said that the event of removing the wire from one solution and immersing into another may elicit the Salicylate release from the coating. It would be argued that this process inadvertently accelerates the Salicylate elution, and that any elution observed was as a result of surface removal. Our results have shown that the mechanism of elution due to Salicylate on the surface is a factor in the release but diffusion from entrapped Salicylate and those bound to the polymer backbone are also key factors. Other potential difficulties of this system are that the diffusion method of elution may be hindered if an equilibrium point is reached in the concentration gradients between the solution and the coating. Along with this, the method of diffusion may provide anomalous results, were large amounts of drug can be released on the back of a prior limited release – this may be representative of the diffusion shifts in the polymer layer. However, as investigators we believed that this was the best available method to test drug elution. The fact that we changed the solution with every time point should have helped ensure that no concentration balance was reached, that diffusion and elution was not hindered by the solution saturation and that the system was representative of the changes that occur in the stent lumen, namely the consistent flow of fluid over the coating layer. Our results have also highlighted that a drug release profile can be observed and large parts of the release style can be accounted for. Further, the method allowed us to make clear distinctions and describe patterns of how the drug release varies with experimental condition changes. The problem with a system using one release vial is that potential saturation and equilibrium could be reached and the sampling and analysis of this required larger sample would be more difficult. The most applicable in-vitro test would involve physiological solution or 'blood' passing over a stainless steel wire coating or through the diameter of a coated stent implanted into an ex vivo artery within a perfusion circuit.

The analysis of the coating thickness, structure and mechanical property was qualitative in nature. These observations provided a basis to make general comparisons between different coating conditions, but no quantitative comparisons could be made between thickness, strength and even malleability and the level or amount of monomer or anion concentrations used.

Variances between some wires produced from the same experimental conditions were discovered and no explanation could be offered for the differences since all parts of the procedure were standardised as far as was possible. The 'current vs time' graphs were very indicative of the resulting coating properties that included the thickness, strength and drug release profile. However, the delivery of potentiostatic voltage in our experimental model means that the voltage was kept constant. Hence, changes in the level of the current must have been triggered by something

different in the electrodes or the solvent. No definite explanations could be found for these differences and hence for the variability in coating properties and drug release profiles observed within the same replicates. However, the findings did highlight the role of current flow in promoting oxidation and polymerisation. This aspect is of key concern if the use of conducting polymers is to occur on a mass scale, because achieving standardisation in medical devices produced is a key aspect of the health and safety recommendations for these devices. A device, not acting in the manner expected, may affect the therapeutic treatment the patient receives detrimentally. Our results could have potentially been affected by confounding factors or other variables not accounted for. Certain factors would have changed as a result of altering the Pyrrole and Salicylate concentration, namely the water content ratio of the solvent and its acidity. It would be instructive for anyone performing a similar study, testing the effect of concentration changes on conducting polymer properties, to measure the acidity and water content of the solution for each sample. Hence, any analysis would involve observing links between change in acidity or water content of the solution and the properties of the coating.

4.9 Conclusion

This study has shown that it is possible to coat a bare metal stent with a conducting polymer and produce drug eluting properties. The study has demonstrated the ability to develop thin and stable coatings on stainless steel metal. The study has also demonstrated the conducting polymer's ability to elute a drug in profiles that are favourable to the agent's mode of action. However, in many of these cases, the developed coating suffered from structural and mechanical weaknesses. The results from our study can act as a basis for further study into the role of monomer concentration and anion concentration on the properties of conducting polymer coatings in drug delivery systems, more specifically in drug-eluting stents. Recommendations have been made for the further study of combinations of the concentrations to yield coatings that meet the design requirements and a further recommendation is made to ensure that these coatings are tested on actual bare metal stents. It would also be interesting to observe the effects of varying a host of other parameters such as the amount of potential applied, the time the potential is applied for, different monomer combinations, other relevant anions/drugs, the use of multiple coating layers, varying the acidic nature of the solvent, changing the solvent's characteristics and even the temperature of synthesis. The possible combinations are numerous and justify further research into conducting polymer technology use in stents, to fulfil the desire to produce enhanced coronary artery stents which may help to improve treatment of coronary heart disease patients.

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