Syntheses, Electrochemistry and Cytotoxicity of Ferrocene-containing Polyaspartamides as Water-soluble Polymeric Drug Carrier/Drug Conjugates

J. C. Swarts

Department of Chemistry, University of the Free State, Bloemfontein, 9300, South Africa

Summary: A general strategy towards the syntheses of water-soluble polymeric drug carriers and their drug conjugates is described. Methods of drug uptake by cells, drug release from the polymeric carrier and the relevance of electrochemistry to drug activity of the ferrocenyl group are highlighted. The advantages of these polymeric systems are demonstrated utilising cytotoxicity results of a polyaspartamide-ferrocenyl conjugate.

Problems associated with small-molecule chemotherapeutic drugs

When one considers metal-containing chemotherapeutic or antineoplastic drugs (the latter are material that have been shown to be capable of killing cancer cells, *i.e.* they are cytotoxic, but are not in clinical use), the drug cis-diamminedichloroplatinum(II) (cisplatin) must surely come to mind. Cisplatin is probably the most successful metal-containing anticancer drugs that was used in recent times.[1] It showed amongst others almost 100% cure rates against ovarian and testicular cancers.[2] However, most if not all anticancer drugs, or potentially useful antineoplastic material, suffer from many negative side effects which either limit or exclude their use in clinical chemotherapy. For cisplatin, these negative side effects include *inter alia* poor aqueous solubility, high toxicity especially to the kidneys and bone marrow,[3] it induces loss of appetite (anorexia)[4] and the metastatic nature of cancer cells quickly leads to the development of drug resistance after continued drug dosage.[5] In addition, cisplatin, like many other chemotherapeutic agents, is itself moderately carcinogenic and can induce, for example, lung cancer in a patient.[6] Further, one must realise that chemotherapeutic agents actually are poisons. The defence mechanism of the body recognise them as such and try to remove them as fast as possible. A high rate of excretion from the body, however, often proves to be very detrimental in chemotherapy. For cisplatin,[7] the 50% lethal dosage applicable to mice is 14 mg/kg body mass of the test animal, the optimum doses is 7 mg/kg but at 3 mg/kg the drug has no effect. Bearing in mind that the biphasic excretion rate of cisplatin from the body is such that 50% of the initial administered

 CCC 1022-1360/00/$ 17.50+.50/0

dose is removed by the reticuloendothelial system within 20 hours, and that 70% of the initial amount of administered drug is excreted within 110 hours,[8] it is obvious that in order to actually obtain a beneficial effect in chemotherapy, an overdose of the cytotoxic agent must be administered to a patient. This explains the many negative side effects associated with chemotherapy. The most important limiting factor in the clinical use of most, if not all, chemotherapeutic drugs is associated with the inability of the drug to distinguish between healthy and cancerous cells.[9] To combat these negative aspects associated with many chemotherapeutic drugs, new antineoplastic material are continuously being synthesised and evaluated,[10] combination therapy has been investigated in the hope of finding synergistic effects,[11] completely new ways of fighting cancer, such as photodynamic cancer therapy,[12] is being investigated, and new methods of delivering an active drug to a cancerous growth are being developed.[13]

Properties of polymeric drug delivery devices

Regarding drug delivery devices that will improve cancer cell specificity of a drug during chemotherapy, what is needed, is a transporting device which actually behaves as a shield or protective envelope into which the drug may be placed. While attached to, or absorbed by this transporting device, the drug should be totally inert in a biological environment. The administered transport device, with the drug attached to it, should then be capable of utilising the bodies central circulation department to be distributed through the body in order to reach and gain access to a cancerous growth without being recognised as undesirable by the bodies' own defence mechanism, the reticuloendothelial system. To make use of the blood to be distributed through the body implies the carrier device must be well water-soluble. The carrier device should further be capable of distinguishing between healthy and cancerous cells, that is, it should be absorbed by cancer cells only, not by healthy cells. Once internalised by cancer cells, the payload of drug must be separated from the carrier and delivered as a free drug in the cancer cell interior. This implies that the bond keeping the drug and carrier together should be biodegradable. The controlled release of the drug inside the cancer cell should in principle serve to activate it and allow the drug to destroy or damage the DNA of cancer cells in a way that is sufficient to cause cancer cell death.

A good candidate for this ideal drug-delivering device is a water-soluble polymeric drug carrier. Some of the properties that should be built into the polymeric drug carrier

includes biocompatibility, water-solubility, it must have a large amount of drug attachment sites which must allow easy drug-polymer coupling reactions without side reactions to generate a biodegradable bond between drug and polymer, it must have a sufficiently large molecular mass to prevent quick excretion from the body (the threshold for elimination *via* the kidneys is *ca.* 70000 g mol^{-1}) yet it must itself be biodegradable to allow ultimate elimination of the spent polymeric carrier from the body after its payload of drug has been delivered to the target site and it must be non-toxic, non-antigenic or non-provocative in any other respect.

Synthetic strategies

Until recently, only a few efforts were made to actually prepare such drug delivery systems. The general strategy to synthesise water-soluble polymeric drug carriers and to covalently anchor a potential drug to it, is shown in Figure 1. The process may also include functionalisation of the drug to allow coupling to the polymeric drug carrier *via* a biodegradable bond. Step reaction polymerisation reactions between monomers of the

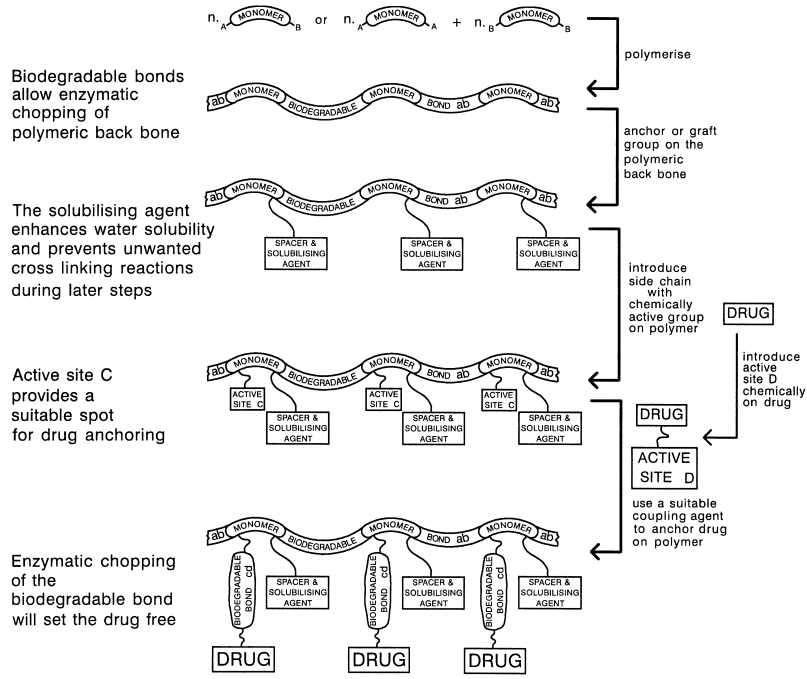
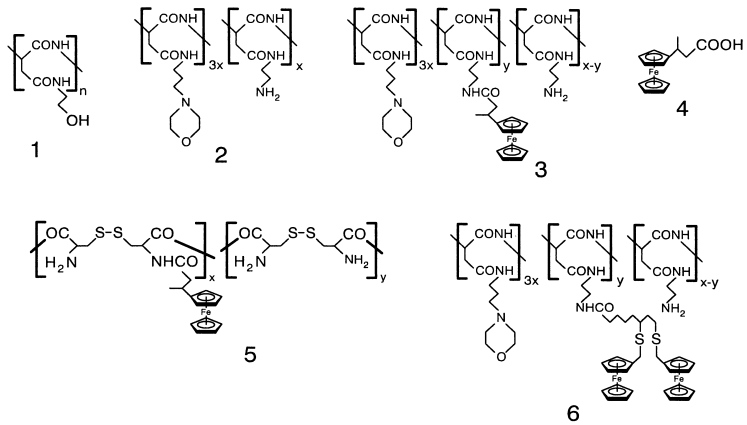


Figure 1. General strategy to synthesise a water-soluble, biodegradable polymeric drug carrier/drug conjugate.

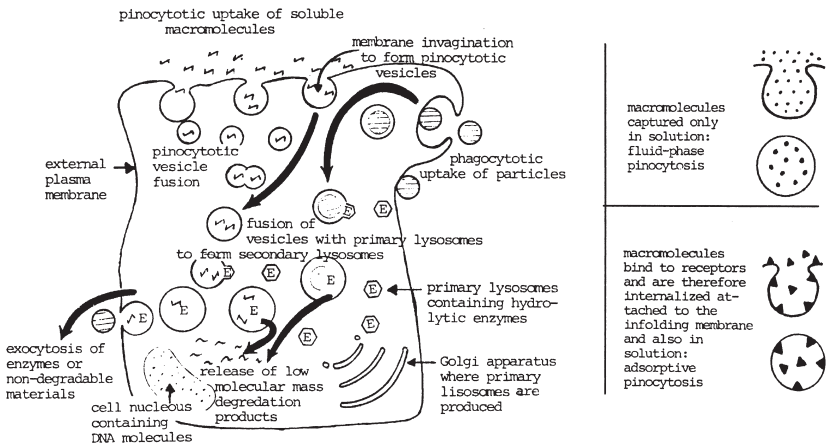
AB or AA and AB type (A and B represent functional groups that are suitable to generate biodegradable -ab- bonds between monomeric units) are particularly useful. The biodegradable -ab- bond may be a peptide (*i.e.* amide), sugar or nucleotide bond, while the spacer group shown in Figure 1 serves to keep active sites on the polymer sufficiently far apart to prevent unwanted side reactions and simultaneously enhances the water-solubility of the polymer. In terms of water-solubility it is instructive to note the influence of carbon atom chain length on the solubility of linear alcohols. Only the C_1, C_2 and C_3 analogues are 100% miscible with water.[14] A crude guideline towards water-solubility is therefore that, where possible, no more than three hydrophobic carbon atoms should be adjacent to each other, both in the main chain (polymer backbone) and polymer side chains, before the next hydrophilic group (e.g. carbonyl, imine, ether etc.) is encountered. Derivatives of aspartic acid are especially suitable as polymeric drug carriers, as they were shown to be so biocompatible, that the alcohol derivative **1** was proposed as a blood plasma expander.[15] Since cancer cells has a higher metabolism rate and grow faster than normal cells, it has a greater demand for energy and the building blocks of tissue, *i.e.* amino acids. To satisfy their need for amino acids during extraordinarily fast growth cycles, cancer cells may show a faster uptake of polyaspartamide derivatives than normal cells. This hypothesis was tested by anchoring the antineoplastic ferrocenyl group onto the polymeric drug carrier **2** to obtain the carrier-drug conjugate **3** utilising techniques borrowed from biochemistry.[16] The coupling agents, N-hydroxybenzotriazole and N-hydroxysuccinimide were found to be lacking in effectivity for this coupling reaction. Coupling under the influence of the former only resulted in a 40% success rate in anchoring 3-ferrocenylbutanoate, **4**, to **2** to



give **3** over 2 days, the latter compound induced 80% effective coupling (that is y = 0.8x) over 20 hours. However, the coupling agent O-benzotriazolyl-N,N,N',N'-tetramethyluronium hexafluorophosphate induced degrees of coupling approaching 90 % within 1 hour at room temperature. When the cytotoxic activity of the polymer/drug conjugate **3** and the free drug **4** was compared, it was found that the antineoplastic activity of the polymer bound drug increased almost one order of magnitude, from a LD 90 value of 500 μg/ml for **4** to 80 μg/ml *drug content* utilising the polymer device **3**.[17]

Cell uptake and drug release

Uptake by cells of polymeric device such as **3** probably occurs by endocytosis, in particular fluid phase pinocytosis as described by Duncan.[9] As shown below (diagram adapted from ref. 9), polymers in solution are internalised into a cell by means of fluid phase pinocytosis. Release of the drug from the polyamide carriers inside the cell occurs by enzymatic hydrolyses of the biodegradable amide (peptide) bonds after fusion of primary lysosomes containing more than 50 hydrolytic enzymes that is generated in the Golgi apparatus with the pinocytotic vesicles containing the dissolved macromolecules. Biodegradable bonds other than the peptide bond may also be used to achieve release of the drug from the polymer as well as digestion of the polymer itself to small-molecule degradation products. These would include bonds that can be cleaved by redox reactions, for example the reduction of the disulphide bond in **5** to thiol groups, or bonds that can be cleaved in an acidic environment. An example of the latter type of bonds is the thio-ether group in **6** that hydrolyses in mild acidic media.[18] These bonds should be



stable at physiological pH (~7.4) but should cleave in cellular environments which often has a pH as low as 5.5. Base-labile or particularly easily hydrolyseable bonds, for example an ester bond, are not desirable if the polymer is to be distributed in the blood. Such very labile bonds may biodegrade (hydrolyse) prematurely, implying drug release may occur before a cancer cell has absorbed the drug carrier with its payload of drug.

Ferrocenyl group antitumor mechanism, relevance to electrochemistry

The mechanism of antitumor activity of the ferrocenyl group is based upon electron transfer.[19] The Fe^{II}-containing ferrocenyl group needs first to be activated by oxidation to a Fe^{III}-containing ferricenium species by redox-active enzymes in a particular body compartment. The ferricenium species then interact with water and oxygen to generate a hydroxy radical. The hydroxy radical then cleaves the DNA strands. Electrochemical and biological studies[17, 19] indicated that only ferrocenyl derivatives with formal reduction potential less than *ca.* 0.216 V *versus* a saturated calomel electrode are active in cytotoxicity experiments. Ferrocenyl oxidation by redox enzymes in the cell becomes thermodynamically impossible in compounds with much more positive formal reduction potentials and the ferrocenyl derivative becomes for all practical purposes inactive.

Acknowledgements

The author acknowledges CANSA, the UFS and the NRF for financial support.

[1] S. E. Sherman, S. J. Lippard, *Chem. Rev.* **1987**, *87*, 1153.
[2] H. J. Wallace, D. J. Higby, in "*Platinum coordination complexes in cancer therapy*", T. A. Conners, J. J. Roberts, Eds., Springer-Verlag, Heidelberg 1974, p.128, 167.
[3] M. Rozencweig, D. D. Van Hoff, M. Slavik, J. Chrisholm, *Ann. Intern. Med.* **1997**, *86*, 803.
[4] J. H. Burchenal, K. Kalaher, T. O' Toole, J. Chrisholm, *Cancer Res.* **1977**, *37*, 3455.
[5] W.Wolf, R. C. Manaka, *J. Clin. Hemotol. Oncol.*, **1977**, *7*, 79.
[6] W. R. Leopold, E. C. Miller, J. A. Miller, *Cancer Res.*, **1979**, *39*, 913.
[7] M. K. Wolpert-DeFillippes, *Cancer Treat. Rep.*, **1979**, *63*, 1453.
[8] J.Drobnik, P. Horacek, *Chem. -Biol. Interactions I*, **1973**, *7*, 223.
[9] R. Duncan, J. Kopecek, *Adv. Polym. Sci.*, **1984**, *57*, 51.
[10] P. Köpf-Maier, H. Köpf, *Chem. Rev.*, **1987**, *87*, 1137.
[11] G. R. Gale, L. M. Atkins, S. J. Meischen, A. B. Smith, E. Walker, *Cancer Treat. Rep.*, **1977**, *61*, 445.
[12] W. M. Sharman, C. M. Allen, J. E. Van Lier, *Drug Discovery Today*, **1999**, *44*, 507.
[13] G. Caldwell, E. W. Neuse, C. E. J. Van Rensburg, *J. Inorg. Organomet. Pol.*, **1997**, *7*, 217.
[14] R. T. Morrison, R. N. Boyd, "*Organic Chemistry*", 6th ed., Prentice Hall, London, 1992, p.217.
[15] P. Neri, G. Antoni, F. Benvenuti, F. Cocola, G. Gazzei, *J. Med. Chem.*, **1973**, *16*, 893.
[16] J. C. Swarts, E. W. Neuse, G. J. Lamprecht, *J. Inorg. Organomet. Pol.*, **1994**, *4*, 143.
[17] J. C. Swarts, D. M. Swarts, E. W. Neuse, C. La Madeleine, J. E. Van Lier, Anticancer Res., **2001**, *21(3b)*, 2032.
[18] A. S. J. Steward, C. N. C. Drey, *J. Chem. Soc., Perkin. Trans. I*, **1990**, 1753.
[19] D. Osella, M. Ferrali, P. Zanello, F. Laschi, M. Fontani, C. Nervi, G. Cavigiolio, *Inorg. Chim. Acta*, **2000**, *306*, 42.