Declaration

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination in any other University.

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ABSTRACT

Presently used anticancer drugs deficiency prompted studies of their possible binding to polymeric structures called carriers which serve as vehicles for transportation of the drug to the target cell. Availability of natural polymers, firstly used, was limited by intrinsic characteristics required by anticancer agents. Synthetic polymers are advantageous as they may be tailored to meet specific structural requirements. They should thus possess desirable biomedical properties such as water-solubility, biodegradability and biocompatibility. They should also bear functional groups for drug binding.

In the present dissertation project, known and novel water-soluble carriers were synthesized and some of them were used for bioreversible binding (anchoring) of antineoplastic agents, such as *methotrexate* and *ferrocene*, to provide conjugates for biomedical application.

Polyaspartamide carriers equipped with hydrosolubilizing and amine-terminated side chains suitable for drug attachment were prepared from poly-D, L-succinimide by a previously developed technique involving successive aminolytic ring-opening steps mediated by amines. Chosen amines include diethylenetriamine (DET), 2, 2'-(ethylenedioxy)diethylamine (EDDA), hydrazine (HY), 1,3-propylenediamine (PDA) and 3-(N,N-dimethylamino)propylamine (DMP).

Other aliphatic polyamide carriers were prepared by polycondensation methods involving 2-hydroxypyridine as a catalyst, diethyl L-tartrate (TART) and diamines ethylenediamine (EDA), EDDA and 4, 7, 10-trioxa-1,13-tridecanediamine (TRIA). The use of diethyl L-tartrate leads to polyamides bearing hydroxyl groups for drug

attachment, while the three diamine monomers enhance polymer solubility and provide intra-chain drug anchoring sites.

Polyamidoamine carriers, possessing intra-chain functional amino and hydroxyl groups for drug binding, were synthesized by a Michael polyaddition reaction of methylene bisacrylamide (MBA) with comonomer 3-amino-1, 2-propanediol (APD) and 3-(N,N-dimethylamino)propylamine (DMP).

All polymeric carriers prepared were fractionated by aqueous-phase dialysis in 12000-14000 molecular-mass cut-off membrane tubing (25000 cut-off for selected polymers) and isolated by freeze-drying. Yields ranged from 10 to 69% and inherent viscosities, $\eta_{inh,}$ from 6 to 29 mL/g. They were then characterized spectroscopically. All polymers dissolved completely in aqueous media, thus fulfilling the requirements of water solubility.

Methotrexate is one of the highly potent anticancer drugs. The literature reveals high folic acid antagonistic properties and high antiproliferative activity against all kinds of cells.

In the present dissertation, a series of water-soluble methotrexate conjugates was synthesized by N-acylation of linear appropriately amine-functionalized polyaspartamide carriers with methotrexate. Acylation was brought about by mediation of O-benzotriazol-1-yl-tetramethyluronium salt (HBTU) as coupling agent.

The anticancer activity of metal compounds has been of major interest in drug research for two decades. The literature shows certain ferrocene-bound conjugates to possess the same high antineoplastic activity against HeLa cells as observed with analogous platinum conjugates.

In the present dissertation, a series of water-soluble *ferrocene* conjugates was synthesized by using the same method as for methotrexate: N-acylation of linear amine-functionalized polyaspartamide carriers with 4-ferrocenylbutanoic acid.

Acylation was here again brought about by mediation of O-benzotriazol-1-yl-tetramethyluronium salt (HBTU) as coupling agent.

All conjugates synthesized were purified by aqueous-phase dialysis and collected by freeze-drying as water-soluble solids. Resulting water-soluble polymer-drug conjugates displayed inherent viscosities, $\eta_{inh,}$ of 5 to 24mL/g, and contained 18-23 % of drug by weight.

In conformance with the project's objective, *methotrexate* conjugates were contributed to a major pool of experimental polymeric antineoplastic agents, to be submitted to the department of Pharmacology, University of Pretoria, for extended cell culture testing to asses their antiproliferative activity.

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CONTENTS

LIST OF ABREVIATIONS

AA: Atomic absorption

AEE: 2-(2-aminoethoxy)ethanol

Asp: Aspartic acid

DCC: Dicyclohexylcarbodiimide

DET: Diethylenetriamine

Detart: Diethyl L-tartrate

DME: 2-(N, N-dimethylamino) ethylamine

DMF: N,N-dimetyhylformamide

DMP: 3-(N, N-dimethylamino) propylamine

DMSO: Dimethylsulfoxyde

EA: Ethanolamine.

DNA: Deoxyribonucleic acid

EDA: Ethylenediamine

EDDA: 2, 2'-(ethylenedioxy)diethylamine

Fc: Ferrocenyl

HBTU: 2-(1H-benzotriazol-1-yl)-1,3,3,3-tetramethyluronium fluorophosphate

His. HCl: histamine hydrochloride

INH: Inherent

MBA: Methylenebisacrylamide

MTX: Methotrexate

NEt_3: Triethylamine

NMR: Nuclear magnetic resonance

O/N: Overnight

PDA: 1, 3-propylenediamine

PSI: Poly-D, L-succinimide

RNA: Ribonucleic acid

RT: Room temperature

TRIA: 4, 7, 10-trioxa-1, 13-tridecanediamine