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AN INTEGRATED PROCESS FOR THE RECOVERY
OF CLINICALLY SIGNIFICANT TRACE PROTEINS
FROM HUMAN PLASMA

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degree of Doctor of Philosophy in Biochemistry

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ABSTRACT

Methods for the preparation of concentrates of factor VIII, factor IX, high purity factor IX, C1 esterase inhibitor, specific immunoglobulin and platelet factor XIII are described. These procedures were developed or modified with the aim of integration into an automated process that would allow sequential recovery of all the clinically significant trace proteins from a single plasma pool. Concomitant recovery of important proteins such as transferrin, alpha-1-antitrypsin and platelet-derived growth factor was considered.

A high-purity factor VIII concentrate heat-treated at 80°C for 96 h was prepared by a process that incorporated heparin fractionation. This method was shown to be suitable for assimilation into an existing regional blood processing laboratory. Several ion-exchange procedures for the recovery of factor IX were evaluated and higher purification of a factor IX concentrate was achieved on a new cellulose-based chromatographic medium. A chromatographic procedure for the preparation of a heat-treated high-purity C1 esterase inhibitor concentrate was described and the performance of a new cellulose-based desalting medium was evaluated in comparison with ultrafiltration. A heat-treated specific immunoglobulin concentrate was prepared from side-stream fractions of an automated chromatographic process for the production of albumin concentrate, and a pilot study for the fractionation of outdated platelet concentrates was carried out with the aim of preparing components of potential therapeutic value.

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ABBREVIATIONS

The standard convention for SI unit abbreviations is followed throughout this thesis.

Other abbreviations:

2D IEP	two-dimensional immunoelectrophoresis
AT III	antithrombin III
C1 INH	complement component 1 esterase inhibitor
CAE	cellulose acetate electrophoresis
CDI	1,1'-Carbonyldiimidazole
CPD	citrate-phosphate-dextrose anticoagulant
CPG	controlled pore glass
CSL	Commonwealth Serum Laboratories
FPLC	Fast Protein Liquid Chromatography
HANE	Hereditary angioneurotic (o)edema.
HETP	height equal to a theoretical plate
HRP	horse-radish peroxidase
I	"conductivity"
IEF	isoelectric focusing
IEP	immunoelectrophoresis
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IV	intravenous
NAPTT	non-activated partial thromboplastin time
NZBTS	New Zealand Blood Transfusion Service
PAGE	polyacrylamide gel electrophoresis
Rocket IEP	rocket immunoelectrophoresis
SDS	sodium dodecyl sulphate
UV	ultraviolet
V_i	interstitial (void) volume
V_r	retention volume

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