CLINICAL TOXICITY OF NICKEL

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To Alan

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LIST OF ABBREVIATIONS

APDC	Ammonium Pyrrolidine Dithiocarbammate
BCR	Bureau Communautaire de Reference
CRF	Chronic Renal Failure
CRM	Certified Reference Material
d.w.	dry weight
D_2-GFAAS	Graphite Furnace Atomic Absorption Spectrometry with Deuterium background correction
EDTA	Ethylendiaminotetraacetic acid
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
GN	Glomerulonephritis
GRI	Glasgow Royal Infirmary
HAS	Human Albumin Solution
i.v.	intravenous
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
ID	Isotopic Dilution
IR	Isotopic Ratio
IUPAC	International Union of Pure and Applied Chemistry
MIBK	Methylisobutylketone
MS	Mass Spectrometry
NIST	National Institute of Standard and Technology
PCA	Principal Component Analysis
PCV	Packed Cell Volume
PPS	Plasma Protein Solution
SEC	Size-Exclusion Chromatography
SRM	Standard Reference Material
TPN	Total Parenteral Nutrition
w.w.	wet weight
Z-GFAAS	Graphite furnace atomic absorption spectrometry with Zeeman background correction

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COMMUNICATIONS AND PUBLICATIONS

M. Patriarca, T.D.B. Lyon, G.S. Fell and B. McGaw. Determination of Ni in human albumin solutions by means of ICP-MS. 5th Surrey Plasma Source Spectrometry Conference, Lumley Castle, 4-7 July 1993.

M. Patriarca and G. S. Fell. Determination of Ni in serum by GFAAS with deuterium background correction. XXVIII Colloquium Spectroscopicum Internationale, Post-Symposium: Graphite atomizer techniques in analytical spectroscopy, University of Durham (UK), 4-7 July 1993.

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SUMMARY

In human health, nickel (Ni) is mainly known as a toxic metal, especially as an allergenic and carcinogenic agent. There is, however, experimental animal evidence of its role as an essential element (Sunderman, 1984).

Nickel toxicity has been widely investigated in relation to occupational exposure. Health hazards for workers exposed to fumes and dusts of this element and its compounds include dermatitis, increased incidence of nasal and lung cancer, chronic diseases of the respiratory tract and chromosomal aberrations (Sunderman, 1984; IARC, 1986; Niebor et al., 1988; IPCS, 1991).

The risk of adverse effects due to Ni toxicity is not confined to the work-place. Because of the widespread use of Ni in modern societies, an increasing number of the general population report allergic dermatitis induced by contact with jewellery, coins and utensils, containing Ni (Edman and Moeller, 1982; McDonagh et al., 1992).

Toxicity may also result from inadvertent exposure to Ni due to medical treatments, such as haemodialysis, intravenous administration of contaminated pharmaceuticals and blood products, and implant of medical devices, such as orthopaedic and orthodontic prosthesis, made of alloys containing Ni (Webster et al., 1980; Olerud et al., 1984; Leach and

Sunderman, 1985; Fell and Maharaj, 1986; Leach and Sunderman, 1987; Gammelgaard and Sandberg, 1989; Landwehr and Van Ketel, 1983; Romaguera and Grimalt, 1985; Vrignaud et al., 1991; Oleffe and Wilmet, 1980; Fine and Karwande, 1990; Wilson and Gould, 1989; Espana et al., 1989; Romaguera et al., 1989; Dunlap et al., 1989; Zoccola et al., 1990; Taylor and Morton, 1991; Burden and Eedy, 1991; Guyuron and Lasa, 1992; Trombelli et al., 1992;). Besides the risk of acute adverse reactions in hypersensitive subjects, the effect of prolonged exposure to low doses of Ni may be of concern. Several reports indicate increased concentrations of Ni in serum of patients undergoing regular haemodialysis (Drazniowsky et al., 1985; Wills et al., 1985; Hopfer et al., 1985; Hopfer et al., 1989; Nixon et al, 1989). Patients with impaired renal function may be at higher risk of Ni retention and accumulation in tissues.

The assessment of risks arising from exposure to low doses of Ni is hampered by the difficulties of Ni analysis at low concentrations and the resultant uncertainty as to the 'true' reference values for a healthy population. Since 1985, the concentrations reported for Ni in serum of unexposed subjects decreased from 1.0 to 0.14 µg/l, (Drazniowsky et al., 1985; Nixon et al, 1989). This suggests that the actual extent of iatrogenic exposure to Ni needs re-evaluation, using more accurate methods.

In this work, I have measured the concentration of Ni in human albumin

solutions used for intravenous administration, which have been produced by different manufacturers, at different times. Additional results for the concentrations of other metals, at various stages of the production process, were also obtained using multielement semiquantitative scanning by inductively coupled plasma-mass spectrometry (ICP-MS) (Chapter 2).

In the last ten years, increasing efforts have been made to limit the contamination of dialysis fluids with metals, present in water, salts and haemodialysis equipment. This was intended to eliminate aluminium toxicity and may have incidentally also reduced Ni contamination. Results for Ni concentrations in the serum of haemodialysed patients are limited and sometimes contradictory, compared to the vast literature on serum aluminium concentrations. The assessment of Ni concentrations has proven more difficult, due to problem of pre-analytical contamination and the lower concentrations present. In Chapter 2 I report an investigation of the serum Ni concentrations in a group of patients undergoing regular haemodialysis and the effect a single dialysis treatment has on serum Ni concentrations in the same subjects.

The understanding of the toxicology of Ni at low doses will benefit from improved knowledge of Ni biochemistry and metabolic pathways. Several studies have been carried out to investigate the metabolism of Ni in man (Nodiya, 1972; Cronin et al., 1980; Solomons et al., 1982; Gawkrodger et

al., 1986; Sunderman et al., 1989) but only two report data on faecal excretion (Nodiya, 1972; Sunderman et al., 1989). There was a large inter-individual variability in the estimates of Ni absorption and excretion. In all the experiments, volunteers ingested Ni, as the naturally occurring mixture of five isotopes, and results could be affected by the contribution of Ni from diet and contamination of samples prior to analysis.

Nickel metabolism has been studied in rats and rabbits using radioisotopes (^{63}Ni, ^{57}Ni) (Onkelinx et al., 1973; Nielsen et al., 1993) but limitations of radiation dosage prevent the application of this technique in man.

The recent development of ICP-MS, which can provide information on the isotopic composition of an element using simplified procedures, offers the opportunity to apply stable isotopes for the study of mineral metabolism in humans on a larger scale.

Different separation procedures were used to remove the mass interferences affecting the determination of the minor Ni isotopes in human albumin solutions, blood, erythrocytes, urine, faeces and tissues. A method was developed, that allowed the determination of three out of five Ni isotopes, one of which was used for isotopic dilution (Chapter 3).

Nickel metabolism was investigated in four volunteers, who ingested a single dose of ^{62}Ni as a tracer. Nickel absorption, distribution and

excretion were determined by analysing plasma, urine and faeces, collected at various time intervals for up to five days (Chapter 4).

The role of Ni in the human environment and the present knowledge on its biochemistry, metabolism, health effects and analytical methods of determination are summarised in Chapter 1.

CHAPTER 1

NICKEL IN THE HUMAN ENVIRONMENT

1.1 INTRODUCTION

Human exposure to Ni, its metabolism, toxicology and analytical biochemistry have been extensively reviewed (Sunderman, 1984; Sigel and Sigel, 1988; IPCS, 1991; Sunderman and Oskarsson, 1991). The aim of this chapter is to provide a concise summary of the present knowledge on these various aspects of the interaction of Ni with the human environment, with particular attention to the most recent findings.

1.2 PROPERTIES, SOURCES, PRODUCTION AND USES OF NICKEL

Nickel, atomic number 28, is the 24th element in order of abundance in the earth's crust and is found almost everywhere in the biosphere. Its average atomic mass is 58.71 and comprises a mixture of five natural isotopes with atomic masses 58, 60, 61, 62 and 64 and relative abundance: 68.077%, 26.223%, 1.140%, 3.634% and 0.926%, respectively (Holden, 1993). Its high melting point (1453°C), excellent

resistance to corrosion and ability to form many alloys make Ni a very useful metal for a variety of applications.

World production of Ni ranges from 700,000 to 900,000 tonnes/year, mainly extracted from pentlandite and laterites deposits in Canada (26%), the USSR (19%) and New Caledonia (12%) (Sigel and Sigel, 1988; IPCS, 1991). Pure Ni is obtained from the mined ore by pyro- and hydro-metallurgical refining processes (IPCS, 1991). Most of the Ni is required for the production of stainless steel and other alloys with high corrosion and temperature resistance.

Nickel alloys and Ni plating are used in vehicles, processing machinery, armaments, tools, electrical equipment, household appliances, cooking utensils, jewellery, coinage, medical devices, like surgical and dental prostheses, computer components and Ni-Cd storage batteries. Pigments for paints, glass and ceramics are based on Ni compounds. Nickel catalysts are used for organic syntheses; petroleum refining; hydrogenation of edible fats, oils and other organic compounds (Sigel and Sigel, 1988; IPCS, 1991).

1.3 ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Atmospheric Ni concentrations in remote areas range from <0.1 to 0.8 ng/m^3. Levels from about 5 to 35 ng/m^3 are found in rural and urban air due to the combustion of fossil fuel such as diesel oil, coal and oil for heat or power generation; the incineration of waste and sewage sludge and emissions from oxidative catalytic converters for automobile exhaust gases (Sunderman, 1986; Boyle and Robinson, 1988). Higher levels are found in larger cities and industrialised areas. Nickel concentrations as high as 3.3 µg/m^3 can occur near Ni smelters (Boyle and Robinson, 1988).

Nickel content in natural water ranges between 0.1 and 0.7 µg/L in sea water, depending on depth, and between 2 and 20 µg/L in fresh water, owing to dissolution of rocks and soil, atmospheric fallout, industrial processes and waste disposal. Drinking water generally contains less than 10 µg/L Ni, but much higher Ni concentrations may be found in areas where Ni is mined. Leakage from the plumbing fittings can increase Ni levels in tap water up to 500 µg/L (Grandjean, 1984). A directive of the Council of the European Communities establishes the limit of 50 µg/L of Ni for water for human consumption (Council of the European Economic Communities. Directive n. 80/778, 15/7/1980).

Nickel can accumulate in plants grown on sewage sludge-treated soils and in vegetation close to Ni-emitting sources. In wildlife, Ni is found in many organs and tissues due to dietary uptake by herbivorous animals and their carnivorous predators. However, there is little or no evidence of biomagnification in the food chain (IPCS, 1991).

Nickel concentration in food are usually below 0.5 mg/Kg fresh weight (Sigel and Sigel, 1988; IPCS, 1991). The highest levels were found in cocoa (10 mg/Kg) and nuts (5 mg/Kg). Also soya products, dried legumes, wheat bran, oatmeal, licorice, tea leaves and sunflower seeds were found to contain Ni concentration > 1 mg/Kg. Vegetables and grains are richer in Ni than animal muscle. Bovine meat contains from 1 to 13 µg/Kg of Ni and bovine liver from 70 to 180 µg/Kg. Shellfish and crustacea may have higher content of Ni compared with other fish.

Daily dietary intake of man varies according to the composition of the diet. In early studies the average Ni intake was estimated to be 168 µg/day for American adults (Myron et al., 1978), 290 µg in the USSR (Nodiya, 1972) and between 200 and 300 µg/day for people living in Western countries (Clemente et al., 1980). In more recent investigations, the Ni daily dietary intake per person was estimated as 150 µg/day in Denmark (Nielsen and Flyvholm, 1984), 150-170 µg/day in Great Britain (Smart and Sherlock, 1987), 90-100 µg/day in Sweden (Becker and

Kumpulainen, 1991) and between 142 and 222 µg/day in Germany (Anke et al, 1991). However, Nielsen and Flyvholm (1984) stressed that Ni intake could reach a level of 900 µg/day with the introduction of certain items such as soya products, dark chocolate or oatmeal in the average diet. Vegetarians may ingest three or four times the average amount of Ni, especially if nuts and chocolate are included in their diet. Regular consumption of canned food may also increase the dietary intake of Ni.

Nickel content of food may be augmented during food processing, particularly in operations involving stainless steel equipment (which may release Ni), the milling of the flour and the hydrogenation of fats and oils using Ni catalysts (IPCS, 1991).

Although the addition of Ni to alloys reduces corrosion, Ni can be released from kitchen utensils, including stainless steel ones. The contribution of these sources, including leaching from water pipes and fittings, to the average daily intake of Ni can occasionally reach 1 mg/day (Grandjean, 1984). In a more recent study, the average contribution from metal cooking ware was estimated as 100 µg/day (Smart and Sherlock, 1987).

Occupational exposure to Ni occurs during Ni production and refining; welding, plating and grinding; in steel plants, foundries and other metal industries (Sunderman, 1984; Sigel and Sigel, 1988; IPCS, 1991). In most

countries, limits of less 1 mg/m^3 have been established for the concentration of atmospheric Ni at work-place (Sunderman and Oskarsson, 1991).

1.4 NICKEL METABOLISM IN MAN

Due to environmental and occupational exposure, Ni enters the human organism and it is found in all body fluids and tissues. The reported Ni content in human biological samples has decreased over the years, owing to improvement of analytical methods and techniques of sampling and storage. However, because of the high risk of contamination and the low levels to be determined, rather few reliable data are available. Some of the most recent available estimates of Ni content in human body fluids and tissues are given in Tables 1.1 and 1.2.

The total content of Ni in the human body has been estimated as 0.5 mg, assuming a mean concentration in tissue of 7 μg/Kg (Bennett, 1985).

Ni uptake occurs mainly through inhalation and ingestion. Penetration through the skin is negligible, but may have important health effects, causing sensitisation and contact dermatitis. The degree of absorption and clearance is influenced by the physico-chemical properties, especially solubility, of the Ni compounds. For the general population, Ni

Table 1.1 Selection of values (mean ± s.d. or range) recently reported for Ni content of human body fluids.

Matrix	Ni, µg/L	N.	Reference
Serum	0.46± 0.26	39	Sunderman et al., 1984
	0.28± 0.24	30	Hopfer et al., 1985
	0.14±0.09	38	Nixon et al., 1989
Urine	2.0± 1.5	34	Sunderman et al., 1986
	0.9 ± 0.11	878	Minoia et al., 1990
Whole blood	1.26± 0.33	30	Sunderman et al., 1984
	0.34± 0.28	30	Hopfer et al., 1985
Bile	2.3 ± 0.8	5	Rezuke et al., 1987
Saliva	1.9±1.0	38	Catalanatto et al., 1977
	0.11± 0.08	12	Hopfer et al., 1985
Sweat[a]	4.0 (1.0-7.6)	10	Pedersen et al., 1985
Milk[a]	(<4.4 -29.6)	16	Mignorance and Lachica, 1985

[a] median (range)

Table 1.2 Selection of values (mean ± s.d. or range) recently reported for Ni content of human tissues and faeces.

Tissue	N	Ni, ng/g Wet Weight	Ni, ng/g Dry Weight	Reference
Lung	15		180±0.105	Seeman et al., 1985
	9	18 ± 12	173±0.094	Rezuke et al., 1987
			170±0.110	Kollmeier et al., 1990
	30	20.2-40.0[a]	107.2-195.4[a]	Raithel et al., 1988
Hilum (left/right)	30	76.7/95.6[b]	387.3/468.3[b]	
Thyroid	8	20±10	141±83	Rezuke et al., 1987
Adrenal	10	26±15	132±84	Rezuke et al., 1987
Kidney	18		34±22	Seeman et al, 1985
	10	9±6	62±43	Rezuke et al., 1987
Heart	9	8±5	54±40	Rezuke et al., 1987
Liver	23		18±21	Seeman et al, 1985
	10	10± 7	50±31	Rezuke et al., 1987
Brain	7	8±2	44±16	Rezuke et al., 1987
Spleen	22		23±20	Seeman et al, 1985
	10	7± 5	37±31	Rezuke et al., 1987
Pancreas	10	8±6	34±25	Rezuke et al., 1987
Hair:[c]				
Japan	457		2.70	Takagi et al, 1986
India	255		0.35	
Bulgaria	36		1.92±0.64	Ward et al., 1987
Italy	100		0.43 ± 0.38	Senofonte et al., 1989
Finger nails[b, c]	95	0.49 (0.04-7.5)		Gammelgaard et al., 1991
Faeces[c]	10	3.3±0.8	14.2± 2.7	Horak et al., 1973
	10	1.5±0.5		Sunderman et al, 1989

[a] lowest and highest value of the median Ni levels observed in the lung lobes

[b] median (range)

[c] µg/g

uptake via inhalation due to environmental exposure is estimated as 0.1-0.7 µg/day and about 0.2 mg Ni per year. This is based on the assumption that the average person living in an urban centre is exposed to approximately 25 ng/m^3 of Ni in air and breathes about 20 m^3 of air per day (ICPS, 1991; Sunderman, 1986; Grandjean, 1984; Niebor et al., 1988). Cigarette smoking contributes from 1 to 12 µg per pack of cigarettes to inhaled Ni (ICPS, 1991; Sunderman, 1986; Grandjean, 1984; Niebor et al., 1988). At the work-place, inhalation of Ni fumes and dusts is the most important route of absorption (dusts of insoluble Ni compounds, aerosols derived from Ni solutions, and gaseous forms containing Ni, usually Ni carbonyl). The relative absorption of Ni from the respiratory tract depends on the particle size, the chemical form and the compartmentalisation within the respiratory tract. Only particles smaller than 10 µm in diameter penetrate the alveolar region of the lung. Solid particles are partially cleared from the respiratory tract by mucociliary action, which in turn results in secondary Ni ingestion. More soluble forms are absorbed into the blood stream, whereas elimination of insoluble Ni compounds take place to a slower rate. Accumulation in lung tissue and regional lymph nodes may occur, although correlation of Ni levels with age has not been definitely confirmed (Kollmeier et al., 1985, 1990; Rezuke et al., 1987; Raithel et al., 1987, 1988).

From 1 to 5% of the Ni ingested with the diet is absorbed from the gastrointestinal tract, but in fasting human volunteers Ni uptake was much higher (27%) (Solomons et al., 1982; Sunderman et al., 1989). Foulkes and McMullen (1986a, 1986b) studied the mechanisms of absorption of Ni and Cd from the rat jejunum. They reported that both Ni and Cd were taken up from the lumen by means of a saturable process, which was inhibited by Zn and the constituents of dried skimmed milk. Unlike Cd, Ni was not retained in the mucosa and was rapidly released into the body, according to a passive process with ion flow occurring in both directions. There was no evidence of the presence of Ni carriers on brush borders or basolateral membranes.

Nickel is transported in plasma bound to serum proteins and low molecular weight compounds. The high molecular weight carriers have been identified as albumin and an α_2-macroglobulin, sometimes called nickeloplasmin. Laussac and Sarkar (1984) have described a binding site of human serum albumin responsible for both Ni and Cu transport. The ulterafilterable fraction of serum Ni is associated with aminoacids, mainly L-histidine, and small polypeptides. The proportion of Ni bound to the different plasma components has not yet been definitely established. The results of experiments carried out on rabbit and human serum, *in vivo* and *in vitro*, showed large variability (Table 1.3).

Table 1.3 Percentages of serum Ni bound to albumin, nickeloplasmin and low molecular weight (LMW) ligands reported in various studies

Reference	Specie	Conditions	Albumin	Nickelo plasmin	LMW ligands
Nomoto et al., 1971	Rabbit		40	44	16
	Man		34	26	40
Asato et al., 1975	Rabbit	*in vivo*			15
		in vitro			36
Nomoto, 1980	Man	*in vivo*		43	
Lukassen and Sarkar, 1979	Man	*in vitro*	95.7	0.1	4.2
Niebor et al., 1988	Man	*in vivo*			24

The values reported for the Ni content of serum and whole blood (Table 1.1), suggest that Ni is not preferentially accumulated in erythrocytes. From the results of *in vitro* experiments, Niebor et al. (1984, 1988) concluded that the presence in serum of high concentrations of albumin and aminoacids prevent the accumulation of Ni in the cellular components of blood. The distribution of Ni between plasma and blood cell types needs to be further investigated.

In animal treated with the radioactive isotope, Ni rapidly appeared in kidney, pituitary, lung, skin, adrenal and ovary or testis. Nickel is found in all human tissues: analysis of autopsy specimens showed the highest concentrations in lung, thyroid and adrenal (Table 1.2).

In experiments on animals, Ni crossed the placenta and was transported to the foetus (IPCS, 1991; Lu et al, 1981; Saillenfait, 1993). The presence of Ni in human foetal tissue has also been reported as indirect evidence of Ni transport through the human placenta (ICPS, 1991; Casey and Robinson, 1978). However, the results are high and their reliability may be questionable.

Excretion of absorbed Ni occurs mainly in urine, probably bound to low molecular weight compounds. Under physiological conditions, Glennon and Sarkar (1982) observed an equilibrium between Ni complexes with albumin and L-histidine in serum, suggesting that the strong affinity of Ni

for L-histidine could explain the rapid excretion of Ni in urine after oral or i.v. administration. From results obtained on the urinary excretion of Ni in workers exposed to almost constant levels of Ni in air, Niebor et al (1988) proposed the existence of a mechanism for Ni reabsorption in the kidney. Rezuke et al. (1987) suggested that biliary excretion may be an additional route of Ni elimination, as they observed Ni concentrations in human bile comparable to those in urine (Table 1.1). The concentrations for Ni content of breast milk and sweat are much higher than urine (Table 1.1). However, very few results are available for these excretory pathways.

1.5 HEALTH EFFECTS

Nickel deficiency: Ni is an essential cofactor for some enzymes isolated from plants and bacteria (Sigel and Sigel, 1988; Anke et al., 1984) and symptoms of Ni deficiency have been reproduced in six animal species: chicks, cows, goats, minipigs, rats and sheep. These animals suffered reduced haematopoiesis, depressed growth and metabolic alterations (Niebor et al., 1988, Anke et al., 1984), when deprived of all dietary Ni, the effects being reversed upon addition of Ni at low concentration into the diet.

Minimal amounts of Ni may be needed for humans as well, although

symptoms of Ni deficiency have not yet been demonstrated in man. However, Ni is found in low concentrations in all human tissues and fluids (Table 1.1 and 1.2) and is transported in human serum bound to specific proteins. The narrow concentration ranges observed for Ni in human body fluids could suggest homeostatic control. Niebor et al. (1988) claimed that reabsorption of Ni occurs in the renal tubular system, probably in the proximal tubule. Early reports described increased concentrations of Ni in body fluids in pathological conditions, such as cases of acute myocardial infarction, unstable angina pectoris, burns, hepatic cirrhosis uraemia and rheumatoid arthritis (McNeely et al., 1971; Khan et al., 1984; Leach et al., 1985; Pedersen and Christensen, 1985), without a known external source of extra Ni. However, the values reported for the control groups in some of these papers are very high in comparison with more recent estimates and the results may be unreliable.

At present, evidence for Ni essentiality in man is far from being conclusive. Due to the very low concentrations involved, contamination problems may easily lead to erroneous results and misleading conclusions.

Nickel toxicity: Acute effects of Ni toxicity have been observed in animals, following i.v. or parenteral administration of Ni chloride. These

include: inhibition of natural killer cell activity and T-cell mediated immune response; hyperglycaemia; nephrotoxicity; hepatotoxicity; acute coronary vaso-constriction and bronchoalveolar hyperplasia (Sunderman and Oskarsson, 1991).

Inhalation of Ni carbonyl ($Ni(CO)_4$) results in acute poisoning, which may lead to pulmonary lesions and death in the most severe cases. Ni carbonyl is a volatile Ni compound, used in the production of high purity Ni (Mond process). Due to its high lipid solubility, Ni carbonyl can cross cellular membranes, is easily absorbed from the lung and diffuses in all organs including brain (IPCS, 1991; Niebor et al., 1988)

Prolonged exposure to vapours of water-soluble nickel salts caused chronic respiratory disease, including asthma, bronchitis and pneumoconiosis, sometimes associated with hypertrophic rhinitis, sinusitis, nasal polyposis and/or perforation of the nasal septum (IPCS, 1991; Sunderman and Oskarsson, 1991; Niebor et al., 1988).

A higher frequency of neoplasms of the respiratory system and nasal sinuses was first recognised in 1928 in workers of a Ni refinery in Clydach (Wales). Since then, a number of epidemiological studies on workers in Ni refineries, smelters and factories confirmed these findings. Increased cancer incidence was mainly associated with operations such as roasting, smelting, sintering and electrolytic separation, that involved exposure to

fumes of metallic Ni and/or dusts of low soluble Ni sulphides and oxides (Sunderman, 1984; IPCS, 1991; Niebor et al., 1988).

Increased risk of carcinomas of the larynx, kidney, stomach and soft tissue has also been observed in certain groups of exposed workers, but the evidence was not conclusive (Sunderman, 1984; IPCS, 1991; Niebor et al., 1988).

At present, in most Western countries, limits of less than 1 mg/m^3 have been adopted for concentration of Ni in air at the work-place (Sunderman and Oskarsson, 1991). This measure of occupational hygiene has successfully reduced the incidence of cancer among workers.

In vivo and *in vitro* studies of the mechanism of Ni carcinogenesis provided evidence that Ni can act as a cancer promoter and enhance the activity of other carcinogenic substances. Nickel forms stable complexes with chromatin and induces lesions in nuclear DNA, including: conformational transitions, infidelity of DNA synthesis, inhibition of DNA synthesis and inhibition of DNA excision repair (Sunderman, 1984; IPCS, 1991; Niebor et al., 1988; Stinson et al., 1992; Chang et al., 1993; Misra et al., 1993). In biological system, complexes with peptides and proteins stabilise the trivalent oxidation state for Ni and the activity of the redox couple Ni(III)/Ni(II) can promote the production of free radicals (Niebor et al., 1988; Wang et al., 1993).

Nickel contact hypersensitivity has been widely documented in both the general population and in a number of occupations in which workers were exposed to soluble Ni compounds (IPCS, 1991; Lidén, 1994). Nickel is generally recognised as the most important cause of contact allergy in the general population. In a recent multicentre survey of 22 European dermatology clinics 19.4% of the patients tested over a period of one year demonstrated Ni sensitivity (Menne' et al., 1991). The incidence of Ni hypersensitivity within the general population appears to depend on cultural habits, such as fashion and availability of Ni containing objects. In Western countries, a marked difference between sexes has been reported by all the investigators. Peltonen (1979) and Pryzstowsky et al. (1979) observed Ni dermatitis in approximately 10% of the women and 1% of the men in samples of population in Finland and the USA, respectively. Nielsen and Menne' (1993) found an incidence of Ni hypersensitivity of 11.1% in Danish women compared with 2.2% for men. Fedler and Strömer (1993) reported Ni hypersensitivity in 15.3% of the women and 5.3% of the men in a sample of Swiss healthy subjects. In Canada, Nethercott and Holness (1990) observed a positive reaction to Ni sulphate in 16.7% of the women and 5.1% of the men in a sample of 1074 subjects with suspected contact dermatitis. However, in Nigeria, approximately the same percentage of women (12.4%) and men (11.7%) were affected by Ni

dermatitis, as wearing necklaces and bracelets was equally fashionable among both sexes (Olumide, 1985). In an early report from Kuwait the ratio women to men for Ni hypersensitivity was 1:3 (Kanan, 1969). This unusual finding was attributed to increased sensitisation of men through watch straps and clothes accessories, whereas, at the time, very few women in Kuwait wore cheap jewellery.

The higher frequency of Ni sensitivity in Western women compared with men has been attributed to higher exposure through costume jewellery and kitchen utensils. Ear piercing has been strongly associated with increased frequency of Ni dermatitis (Boss and Menne', 1982; Larsson-Stymne and Widström, 1985; McDonagh et al., 1992; Nielsen and Menne', 1993; Räsänen et al., 1993). In a recent study, Nielsen and Menne' (1993) reported that both the association of age (inverse) and female sex (direct) with increased frequency of Ni positive patch tests disappeared when data were adjusted for the effect of ear piercing.

Sensitisation occurs after localised exposure of the skin to Ni ions, that penetrate the skin and conjugate with proteins to form the antigen. Microvescicular hand eczema is the most common clinical manifestation of Ni dermatitis. After the first outbreak, Ni hypersensitivity is likely to last a lifetime and affect quality of life and ability to work.

Besides allergic reactions at the site of contact, generalised secondary

eruptions may occur without known contact with Ni. The response to oral challenge with Ni salts gave contradictory results (Cronin et al., 1980; Gawkrodger et al., 1986), whereas the ingestion of a diet naturally high in Ni for 4 days induced an exacerbation of hand eczema (IPCS, 1991). Although some patients benefited of the reduction of dietary intake of Ni (Veien et al, 1993; Atakan et al., 1993), others reported lowered degree of allergy following repeated administration of oral doses of Ni sulphate (Sjovall et al., 1987; Santucci et al., 1988, 1994).

At present, owing to the widespread use of nickel in a variety of commodities of everyday use, an increasing number of the general population may be at risk of Ni sensitisation and little can be done to improve this pathological condition. Both reduced Ni intake and repeated Ni oral administration have been reported to produce some benefit in some of the patients, but the mechanisms responsible for these controversial responses are not known. The understanding of Ni dermatitis could profit from improved knowledge of Ni metabolism in healthy and sensitive subjects.

1.6 ANALYTICAL METHODS OF NICKEL DETERMINATION IN BIOLOGICAL MATERIALS

The investigations of Ni metabolism and toxicity requires the determination of Ni levels in biological materials. Since in most of the specimens of interest, the concentrations to be analysed are low and the risk of contamination is high, achieving reliable results is still a difficult task.

The reliability of the analytical measurement alone is not enough to assure the reliability of overall results. Procedures of sample collection, sampling and storage of the samples should be carefully designed and all possible sources of contamination considered and kept under control. Sample pretreatment should be kept to a minimum and carried out under clean conditions, preferably within a laminar flow workstation. Several aspects of the analysis of Ni in various biological materials have been reviewed in detail (IUPAC, 1980; Stoeppler, 1984a; Stoeppler, 1984b; Seiler, 1988; IUPAC, 1994).

Of the techniques available for the determination of trace elements, graphite furnace atomic absorption spectrometry (GFAAS), differential pulse adsorption voltammetry (DPAV) and inductively coupled plasma mass spectrometry (ICP-MS) are the most sensitive. Flame atomic

absorption spectrometry, inductively coupled plasma atomic emission spectrometry and neutron activation analysis can only be used for high concentrations or after enrichment procedures. Detection limits for various techniques are reported in Table 1.4.

At present, GFAAS is the technique of choice for the analysis of Ni in most biological materials. This method is simple, rapid and requires a minimal manipulation of the sample, thus reducing the risk for contamination. Detection limits as low as 0.45 µg/L for urine, 0.1 µg/L for whole blood and serum and 10 ng/g dry weight for tissues, food and faeces have been reported (Sunderman et al., 1984, 1985, 1986). However, according to a recent report, Ni concentrations in serum of healthy subjects appear to be lower than 0.1 µg/L and even more sensitive methods are needed.

Differential pulse adsorption voltammetry offers the highest sensitivity for the determination of Ni (Table 1.4). Accumulation of Ni at the working electrode/solution interface is obtained by adsorption in the presence of a suitable complexing agent such as dimethylglyoxime. The main disadvantage of this procedure is the need for complete mineralisation of biological samples, to avoid interferences with the adsorption process. Since typical Ni content of mineralised blank solutions is about 0.1 ng/ml, this step dramatically reduces the actual detection limits for the

Table 1.4 Detection limits reported in literature for different analytical techniques for the determination of Ni in aqueous solutions.

Method	Detection limit, ng/ml	Reference
FAAS	30	Perkin-Elmer Operator's Manual
RNAA	10	Nuclide: ^{65}Ni, Stoeppler, 1984
ICP-AES	1-5	Que Hee et al., 1985
GFAAS	0.15	This study, ch. 2
Z-GFAAS	0.06	Nixon et al., 1989
ICP/MS	0.03	Horlick et al., 1987
DPAV	0.001	Ostapczuk, 1983

DPAV = Differential Pulse Adsorption Voltammetry
FAAS = Flame Atomic Absorption Spectrometry
GFAAS = Graphite Furnace Atomic Absorption Spectrometry
ICP-AES = Inductively Coupled Plasma -Atomic Emission Spectrometry
ICP/MS = Inductively Coupled Plasma -Mass Spectrometry
RNAA = Radiochemical Neutron Activation Analysis
Z-GFAAS = GFAAS with Zeeeman background correction

determination of Ni in biological materials by this procedure.

Recently, techniques based on mass spectrometry (MS), such as gas chromatography/MS and inductively coupled plasma/MS have been applied to the determination of Ni in serum and urine (Aggarwal et al., 1989; Vaughan and Templeton, 1990; Xu et al., 1993). Although the sensitivity of these procedures may not be optimal and sample pretreatment to overcome matrix interferences may appear cumbersome, the development of such methods which are able to detect the isotopic composition of the sample is important for the use of stable isotopes in both analytical and metabolic investigations.

CHAPTER 2

CLINICAL TOXICITY OF NICKEL

2.1 INTRODUCTION

Due to the widespread uses of Ni and Ni alloys, patients undergoing invasive medical treatments may be at risk of adverse effects from exposure to Ni. Acute intoxication and severe allergic reactions occurred after haemodialysis with fluids inadvertently contaminated with Ni (Webster, 1980; Olerud, 1984). Internal exposure to Ni contained in implants, such as cardiac pacemakers, orthodontic and orthopaedic prostheses, caused allergic reactions and sometimes required further surgery to remove and substitute these items with others made with Ni-free alloys (Fisher, 1977; Landwehr and Van Ketel, 1983; Waterman and Schrik, 1985; Fernandez et al., 1986; Burden and Eedy, 1991; Trombelli et al., 1992; Guyuron and Lasa, 1992; Lowey, 1993). Even the use of a stainless steel needle induced the development of contact dermatitis in a sensitive subject (Romaguera and Grimalt, 1985).

Patients with chronic renal failure maintained on haemodialysis have

higher concentrations of Ni in serum (Drazniowsky et al., 1985; Wills et al., 1985; Hopfer et al., 1985; Hopfer et al., 1989; Nixon et al., 1989) and subjects who had undergone total hip replacement had increased urinary excretion of Ni, Co and Cr due to corrosion and leakage from the prostheses (Hennig et al., 1992).

Nickel concentrations in serum and urine were increased in patients with chronic alcoholism treated with disulfiram. This effect was attributed to chelation of dietary Ni by diethyldithiocarbammate, a metabolite of disulfiram, and enhanced gastrointestinal absorption of this lipophilic Ni complex (Hopfer et al., 1987).

Considerable amounts of potentially toxic metals, including Ni, may contaminate pharmaceuticals, nutrient solutions and blood products for intravenous (i.v.) injection (Milliner et al., 1985; Leach and Sunderman, 1985; Fell et al., 1986; Fell and Maharaj, 1986; Leach and Sunderman, 1987; Diver et al., 1988; Köppel et al., 1988; Gammelgaard and Sandberg, 1989).

Iatrogenic exposure to Ni clearly poses a risk for hypersensitive subjects, but even if clinical signs of Ni toxicity do not appear, the long term consequences of internal exposure to Ni are not known. Since Ni is mainly excreted through the kidney, the elderly and other subjects with reduced renal function, including pre-term babies with immature kidneys,

may accumulate Ni in tissue with possible adverse effects.

This study was aimed to contribute to the understanding of the possible sources of Ni exposure within medical care and focused on two points: the assessment of the contamination with Ni of human albumin solutions (HAS) for i.v. administration and the evaluation of hypernickelaemia in haemodialysed patients.

2.2 NICKEL AND OTHER TRACE METALS IN HUMAN ALBUMIN SOLUTIONS

Human albumin solutions were originally developed as an alternative to blood or reconstituted dried human plasma for the treatment of casualties during the second World War. Since then, they have been widely used in medicine. The conventional therapeutic indications for albumin administration are (McLelland, 1992):

- to replace plasma volume (intravascular expansion replacement) and achieve target values of osmotic colloid pressure or albumin concentration (i.e. in patients with trauma or undergoing major elective surgery);
- to reduce the loss of fluids and proteins observed in burns owing to

prolonged increase in microvascular permeability;

- for extracorporeal circulation during cardiac surgery;
- to achieve diuresis in patients with nephrotic syndrome resistant to diuretics;
- to replace massive protein loss in cirrhotic patients being treated with abdominal paracentesis.
- for therapeutic plasma exchange.

Plasma exchange, i.e. the selective removal of the patient's plasma and its replacement with donor's plasma or another suitable colloid, has been applied in a number of diseases to remove or reduce the concentration of some pathological factors in blood. Table 2.1 lists diseases for which plasma exchange has proven beneficial and those for which clinical advantages are uncertain (Shumak and Rock, 1984). Human albumin solutions or purified plasma fraction are often used as the replacement fluid instead of donor's plasma, to avoid the risk of hepatitis and other viral infections. Exchanges in which the volume replaced approaches the patient's plasma volume are repeated four or five times within seven to ten days for effective therapy. In such cases, patients may receive from 10 to 15 litres of HAS (Shumak and Rock, 1984).

In 1985, Milliner et al. observed that HAS were heavily contaminated with aluminium and could pose a risk for patients with renal dysfunction.

Table 2.1. Disorders commonly treated with plasma exchange (PE), with proven or uncertain efficacy (Shumak and Rock, 1984).

Well- established indications for PE:

Hyperviscosity syndrome
Cold antibody-type autoimmune haemolytic anemia
Post-transfusion purpura
Factor VIII antibody unresponsive to Factor VIII therapy
Myasthenia gravis
Refsum's disease
Goodpasture's syndrome

Indications for PE needing further assessment:

Prevention of hemolytic disease due to Rh antibody
Thrombotic thrombocytopenic purpura
Idiopathic thrombocytopenic purpura
Cryoglobulin-induced disease
Preparation for bone-marrow transplantation
Familial hypercholesterolemia
Acute and chronic relapsing Guillain-Barre' syndrome
Multiple sclerosis
Rheumatoid arthritis
Systemic lupus erythematosus
Rapidly progressive glomerulonephritis
Renal-allograft rejection
Biliary cirrhosis

Human albumin solutions are often administered to patients with renal disease, to expand intravascular volume in patients undergoing haemodialysis and to promote diuresis in subjects with severe nephrotic syndrome. A variety of disorders that benefit from plasma exchange therapy are associated with renal insufficiency, including cryoglobulinemia, systemic lupus erythematosus, Goodpasture's syndrome, rapidly progressive glomerulonephritis, thrombotic thrombocytopenic purpura and renal-allograft rejection (Milliner et al., 1985).

Fell et al. (1986) reported Al contamination of pharmaceuticals, nutrients and blood products used for i.v. administration and Maharaj et al. (1987) described Al bone disease in patients receiving contaminated HAS during plasma exchange therapy.

Leach and Sunderman (1985) observed that HAS is also prone to contamination with considerable amounts of Ni, due to the high affinity of albumin for this metal. Sunderman (1983) strongly recommended 5 µg/L as the maximum allowable concentration of Ni in common fluids for i.v. injection and 10 µg/L for HAS and solutions containing aminoacids, since acute allergic and cardiovascular reactions may occur after i.v. Ni infusion.

Fell and Maharaj (1986) described widely varying concentrations of

potentially toxic metals in both HAS and other colloid solutions. They found values 10 to 1000 times higher than normal serum reference values for Al, Cr, Mn, Ni and Fe; marked depletion of Mg, Cu, Zn and Se; and an excess of Ba and Sr. In a following paper Diver et al. (1988) reached similar conclusions analysing the content of trace elements present in two commercial plasma protein and solutions, although the levels of contamination and depletion were rather different for the two products. This considerable contamination was attributed to the 'scavenging action' of proteins in contact with stainless steel surfaces during the production stages, impurities of the chemicals used during the manufacturing process and leakage from metal and rubber closures of the containers (Fell and Maharaj, 1986).

Köppel et al (1988) investigated the influence of three weeks of daily HAS infusion on metal blood concentrations of eight critically ill patients with acute renal failure, protein catabolism, ventilator therapy and haemodialysis treatment. They reported increased blood Ni concentrations and computed that the Ni dose due to HAS infusion ranged from 5 to 70 µg/day, i.e. higher than the estimated amount absorbed daily from the diet by healthy subjects. Nickel and chromium concentrations in HAS were also increased to 5 and 8 mg/L, respectively, after 24 h contact with the metal cannulas sometimes used for infusion.

Following the earlier reports on the Al content of HAS, efforts have been made to identify the sources of metal contamination during the manufacturing process. The impurities contained in some of the reagents used, such as sodium hydroxide and caprylic acid, and the depth filtration stages appeared as the principal causes of the increased concentration of some trace metals in 5% HAS (Maharaj, 1986; Gammelgaard and Sandberg, 1989). Gammelgaard and Sandberg (1989) reported that depth filtration steps where in some cases performed with filters coated with Al compounds. In addition, both Al and Ni levels showed a further dramatic increase during the concentration process of albumin from 5% to 20%.

In most cases, actions were taken to reduce metal contamination. At the Edinburgh Protein Fractionation Centre, contaminated reagents were identified and substituted or purified, filters were washed with citrate solutions before use and a further step of ultrafiltration was introduced, to selectively remove contaminant metals both as ions and bound to low molecular weight compounds. These changes proved successful for the substantial reduction of the concentrations of Al, Mn and Cr to less than 20% of the initial values, but most of the Ni (75%) was retained because of the high affinity for albumin. Therefore, Ni remained a possible hazard for patients receiving HAS (Maharaj, 1986).

Following these early investigations, improved technology for metal

contamination control has been adopted by manufacturers, such as the substitution of stainless steel with plastic equipment whenever possible. Therefore, there was a need to assess the present concentration of Ni and other metals in commercially available HAS and verify the sources of potential contamination during the manufacturing process. This was accomplished by analysing a number of HAS of different origin and studying the profile of selected trace metals by means of semiquantitative ICP-MS in HAS and in samples from various stages of the production process.

2.2.1 Experimental

Blood products: Samples of human plasma protein solutions (PPS, 85% albumin) and human albumin solutions (HAS) were kindly donated by manufacturers (Protein Fractionation Centre, Scottish Blood Transfusion Service, Edinburgh, Scotland; Farmabiagini, Castelnuovo di Garfagnana, Italy; Istituto Sclavo, Siena, Italy) or otherwise obtained from the Glasgow Royal Infirmary (GRI) pharmacy. Other human blood products (Factor VIII, Factor IX and immunoglobulin) were also obtained from the GRI pharmacy.

Samples from various stages of the production process of PPS at the Protein Fractionation Centre were kindly provided by Dr W. McBey. Selected plasma proteins are obtained by cold/ethanol fractionation. A simplified scheme of the purification process and the points where samples were taken for analysis is shown in Fig. 2.1.

Nickel analysis: Ni determinations were carried out by graphite furnace atomic absorption spectrometry (GFAAS), using a Perkin-Elmer atomic absorption spectrophotometer, mod. 1100, with deuterium arc background correction, equipped with an AS-70 autosampler. Graphite tubes were pyrocoated. Signals were recorded as peak area by a built-in computerised system. Instrumental conditions were as follows: lamp current: 15 mA; wavelength: 232.0 nm; slit: 0.2 nm; integration time: 4 s. The graphite furnace programme is reported in Table 2.2. The injection volume was 50 µl. All samples were diluted 1+2 with a diluent solution containing 1% v/v HNO_3 and 0.25% Triton X-100. Aqueous Ni solutions were used for calibration. Solutions containing more than 200 g/L of albumin were diluted 1+4 and longer drying and ashing steps were used. Analysis of solutions with high Ni content was performed using smaller injection volumes (10-20 µl) and a less sensitive wavelength (301.5).

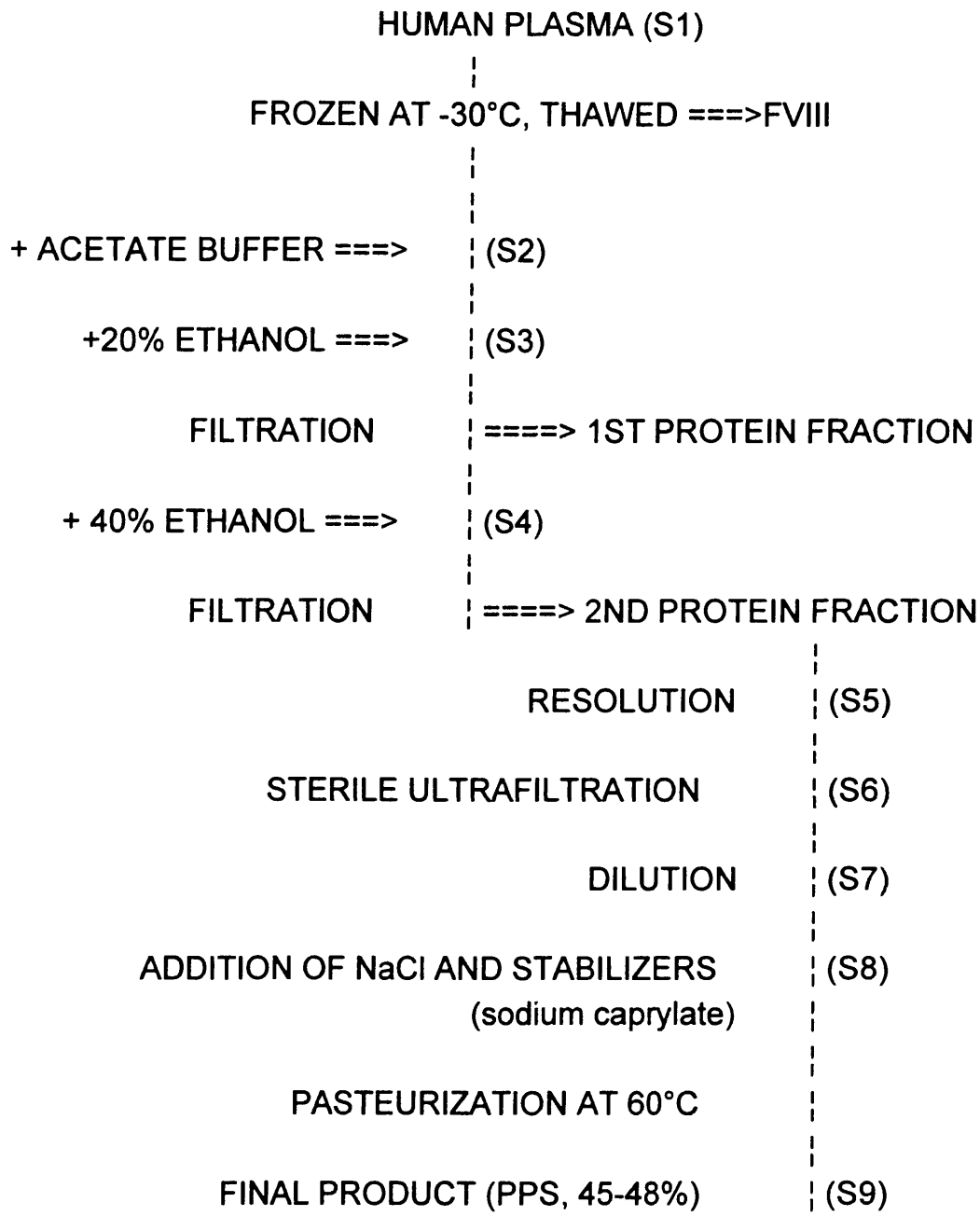

Fig. 2.1 Scheme of the production of PPS at Edinburgh Protein Fractionation Centre and points where samples were withdrawn (S1 to S9)

Table 2.2 Furnace programme for the determination of Ni in HAS by GFAAS

STEP	1	2	3	4	5	6
TEMP, °C	130	180	700	1200	2600	2700
RAMP TIME, s	5	25	10	10	0	1
HOLD TIME, s	10	5	20	20	5	2
READ					on	
GAS FLOW, ML/MIN	300	300	300	300*	0	300

* Gas flow =0 for the last 5 s in this step

ICP-MS analysis: Multielemental semiquantitative determinations were performed on some of the samples by ICP-MS using a PLASMAQUAD instrument (VG Elemental, Winsford, Cheshire, UK) at the Scottish Universities Research Reactor Centre. All specimens were diluted 1+9 with 1% HNO_3. Indium (50 µg/L) was used as internal standard. All measurements were corrected for blank using a 1% HNO_3 solution.

Control of contamination: due to avid binding of Ni by albumin solutions, precautions were taken during the whole analytical procedure to avoid contamination. All plastic ware, including autosampler cups, were soaked in 20% HNO_3 overnight and rinsed six times with purified water. Micropipette tips were washed with 20% HNO_3 and rinsed three times with water before use. Pretreatment of samples expected to have low concentrations of Ni were carried out in a laminar flow hood. Nitric acid used for analysis (Aristar, BDH) was further purified by subboiling in PTFE bottles.

2.2.2. Results

Analytical performance of GFAAS method: the detection limit (three times the value of the SD of 12 measurements of a blank solution) was 0.9 µg/L. The slopes of calibration curves obtained with aqueous and HAS based standards did not differ (y=1.4 + 1.69x for aqueous standards *versus* y=1.6 + 1.67x for HAS based solutions). Analytical recovery of Ni added to a 20% HAS containing 7.3 µg/L Ni, was 102 ± 5%. Within-day and between-day precision, obtained from replicate analysis of a 5% HAS containing 13.6 µg/L Ni, were 3% (n = 8) and 6% (n = 10), respectively.

Contamination of HAS with Ni and other metals: The Ni concentrations in HAS produced over a period of time by various manufacturers are reported in Table 2.3. Table 2.4 lists the Ni content of other blood products. Figure 2.2 shows the dramatic change in Ni concentration in HAS produced in the same centre from 1988 to 1992. In Figure 2.3 the results obtained in this study are compared with those of earlier investigations.

Semiquantitative information on the concentration of other metals in samples of PPS and HAS produced by different manufacturers and in different countries (Scotland, England and Italy) are reported in Table 2.5.

Table 2.3 Concentration of Ni and Co observed in different brands of HAS.

CODE	BRAND	BATCH N.	EXPIRE DATE*	ALBUMIN g/L	N.	Ni µg/L	Co µg/L
H	BPL		FEB 91	45	1	97.8	5.3
B	IMMUNO		JAN 91	43	1	7.4	0.8
C	IMMUNO		AUG 92	43	1	13.8	
D	ARMOUR		NOV 91	50	1	8.3	
G	ARMOUR		MAG 92	50	1	9.7	
E	SBTS, PPS		APR 91	45	1	370	13
A1	SBTS, PPS		JUN 91	45	1	430	17
F	SBTS, PPS		JUL 91	45	1	65	1.1
A	SBTS, PPS		AUG 91	45	1	20.4	0.7
I	SBTS, PPS		OCT 91	45	1	13.5	0.4
L	SBTS, PPS		NOV 94	45	1	13.6	0.8
ED24	SBTS, PPS		SEP 96	45	1	5.2	
ISS1-3	FARMAITALIA	922617	JUL 95	50	3	8	
ISS4-6	FARMAITALIA	924004	JUL 95	50	3	8.6	
KAG	KABI (Germany)		DIC 87	200	1	59	0.9
KAS	KABI (Sweden)		MAR 87	200	1	33	
SHA1	SBTS		SEP 88	200	1	1020	37
SHA2	SBTS		DEC 92	200	1	28	
SHA3	SBTS		JAN 93	200	1	33	
ZEN	BIO-PRODUCTS LAB		JAN 95	200	1	7.3	
ISS7-9	FARMABIAGINI	923324	JUL 95	250	3	40.6	
ISS10-12	FARMABIAGINI	923323	JUL 95	250	3	39.5	
ISS13-14	SCLAVO	AS2=411	APR 95	200	3	41.9	
ISS7-9	SCLAVO	AS2=412	APR 95	200	3	43.7	

BPL = BLOOD PRODUCT LAB

SBTS = SCOTTISH BLOOD TRANSFUSION CENTRE

Table 2.4 Nickel concentration in other human blood products for intravenous injection.

BLOOD PRODUCT	BRAND	EXP. DATE	Ni, µg/L
IMMUNOGLOBULIN	SBTS	JUN 93	10
ANTIHAEMOPHILIC FACTOR	SBTS	JUL 93	32
FACTOR IX CONCENTRATE	SBTS	MAY 93	8

SBTS = SCOTTISH BLOOD TRANSFUSION CENTRE

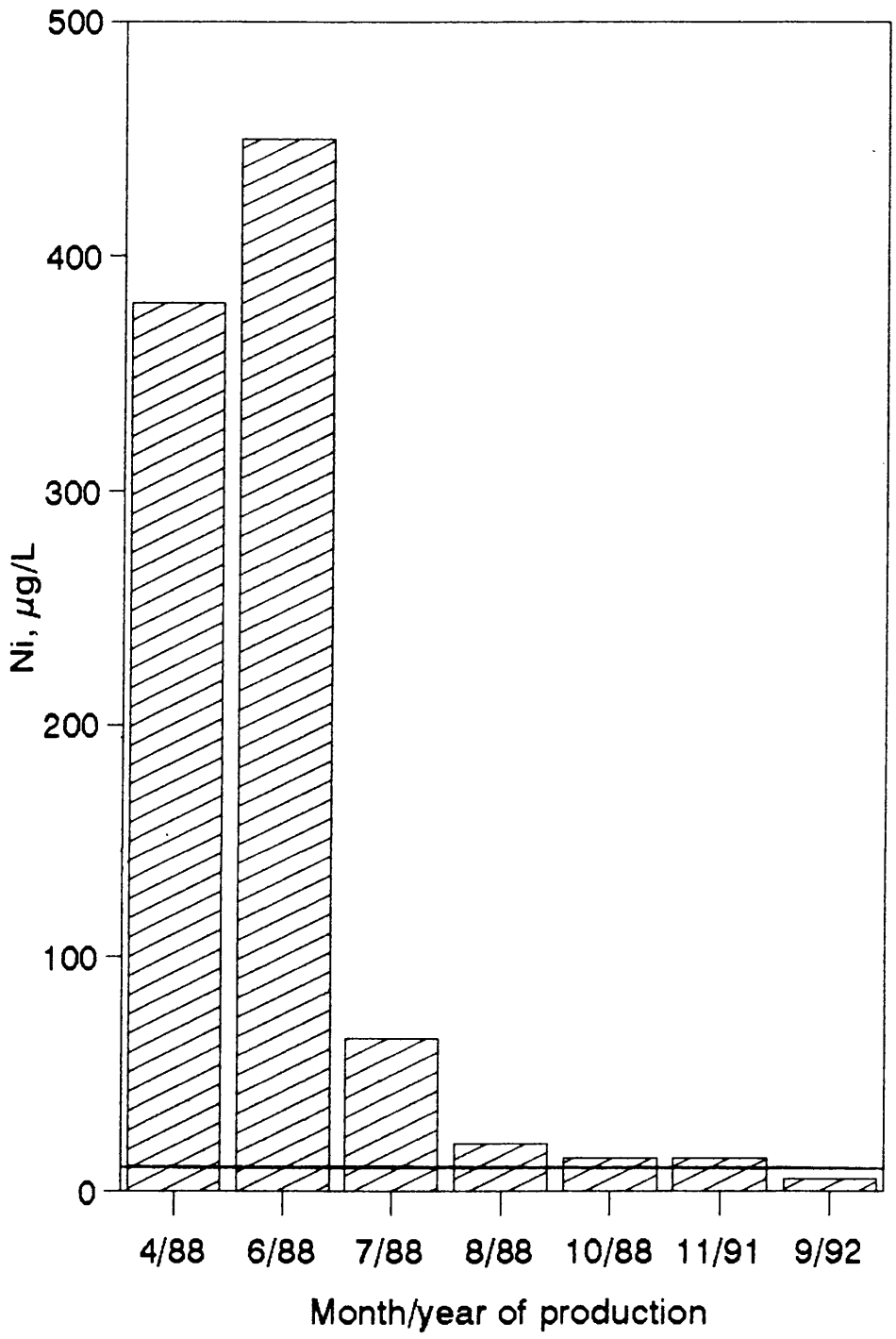

Fig. 2.2 Ni content of HAS (5%) produced at the Edinburgh Protein Fractionation Centre between 1988 and 1992 compared with the suggested limit of 10 µg/L (—).

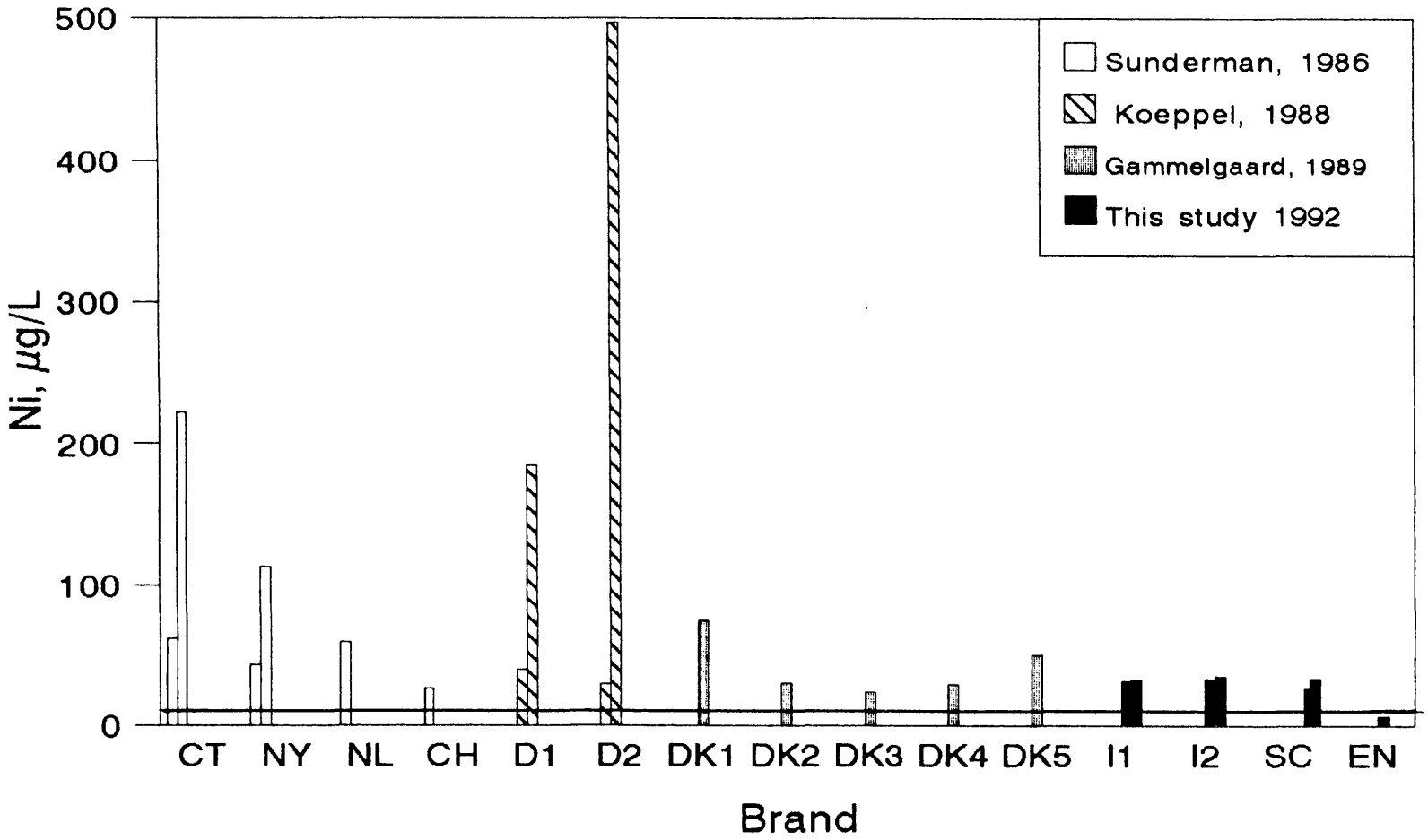

Fig. 2.3 Ni content (min and max) observed in different brands of 20% HAS produced in various countries between 1986 and 1992 compared with a limit of 10 µg/L (—)

Table 2.5 Content of Ni and other metals in PPS and HAS with different protein concentration and from different origin. All data obtained by semiquantitative ICP-MS, except Ni (GFAAS).

COUNTRY	SCOTLAND	ENGLAND	ITALY					
TYPE	PPS 4.5%	HAS 20%	HAS 5%		HAS 25%		HAS 20%	
PRODUCER	SBTS	BioProducts	Farmabiagini		Farmabiagini		Sclavo	
			A	B	A	B	A	B
Al, µg/L	33	244	31	38	133	544	82	185
Ba, µg/L	29	132	8	9	56	62	51	62
Br, µg/L	39	10	13	13	-	-	-	-
Cu, µg/L	239	275	64	70	329	354	580	580
Ni, µg/L	13.6	7.4	7.9	8.5	41.4	41	41.6	43.9
Rb, µg/L	-	-	1	1	5	-	10	15
Sr, µg/L	8	63	6	7	31	21	15	26
W, µg/L	25	115	31	25	108	123	118	92
Zn, µg/L	294	293	256	237	1376	1428	2527	3230

A, B = different batches

The variations of the metal content in the various stages of the production process of PPS at Edinburgh Protein Fractionation Centre are reported in Table 2.6 and Figs. 2.4-2.12. Since Ni and other elements are strongly bound to proteins, the variations of the total protein content at stages of the process are reported for comparison.

2.2.3 Discussion

Results presented in Tables 2.3 and 2.4 confirm the contamination of HAS and other blood products with Ni. Some of the oldest batches contained Ni concentrations as high as 1020 µg/L and there was large variability among manufacturers.

The Ni content of PPS produced at the Edinburgh Fractionation Centre from 1988 and 1992 showed an impressive reduction (Fig. 2.2), although only in 1992 did it reach the limit of 10 µg/L recommended by Sunderman (1983) for solutions for i.v. injection containing albumin. The improvement of the quality of HAS now commercially available is further highlighted by the comparison of results obtained in this study for 20% HAS with those of earlier investigations (Fig. 2.3). The Ni content of batches of 20% HAS produced in both Scotland and Italy in 1992 was close to 40 µg/L. This value, adjusted for concentration, was comparable to the amount of less

than 10 µg/L found in 5% HAS from the same producers. In earlier reports, Ni content ranged from 24 to 497 µg/L. However further reduction of Ni concentration is possible, since a sample of HAS produced by Bioproducts Lab (England) contained only 7.4 µg/L of Ni.

Preliminary results were obtained by ICP-MS on the concentration of Co, also a constituent of stainless steel, in a few samples of HAS. Cobalt concentrations were high in samples with very high concentrations of Ni and decreased to less than 1 µg/L in samples where Ni concentrations were also reduced. This may suggest a common origin for at least some of the Ni and Co contamination (Table 2.3).

The concentration of other metals, determined by semiquantitative ICP-MS, was very variable for samples of different origin and different content of albumin (Table 2.5). Semiquantitative analysis by ICP-MS provides qualitative information over a range of selected elements with an accuracy within 30% (Vaughan et al., 1991). Aluminium values ranged from 31 to 38 µg/L for samples containing 5% of albumin and from 82 to 580 µg/L for samples containing more than 200 g/L of albumin. Also Ba, Sr and W concentrations were increased, whereas essential elements such as Br, Cu and Rb were depleted. Zinc was lower than 0.3 mg/L in some of the samples but as high as 3 mg/L in HAS of Italian origin.

During the manufacturing process, Ni concentration (Table 2.6,

Table 2.6 Concentrations of total protein (TP) and metals in stages of PPS production. Metal concentrations were determined by semiquantitative ICP-MS unless otherwise specified.

Stage	TP g/L	Ni° µg/L	Al* µg/L	Ba µg/L	Br µg/L	Cu µg/L	Rb µg/L	Sr µg/L	W µg/L	Zn µg/L
S1	56	1.5	22	5	211	633	116	40	44	670
S2	56	4.1	16	5	221	656	111	40	23	677
S3	30	1.5	-	3	114	550	84	29	15	564
S4	14	5.8	280	72	69	55	32	100	12	369
S5	89	9.6	294	44	48	694	18	87	64	288
S6	107	8.3	20	20	30	505	-	10	40	208
S7	51	3.7	-	9	29	258	-	5	26	111
S8	45	3.3	3	7	35	239	-	5	27	103
S9	47	5.1	22	10	45	256	2	5	41	111

° GFAAS

* ICP-AES

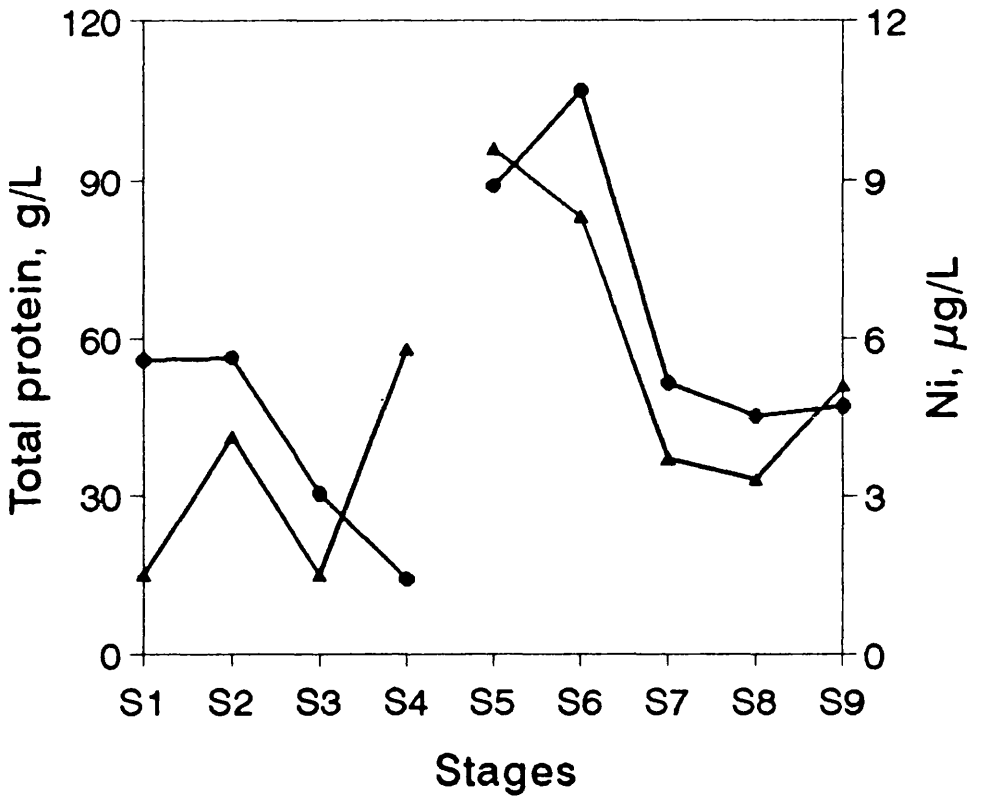

Fig. 2.4 Concentration of Ni (triangles) and total protein (circles) in various stages of the production of PPS.

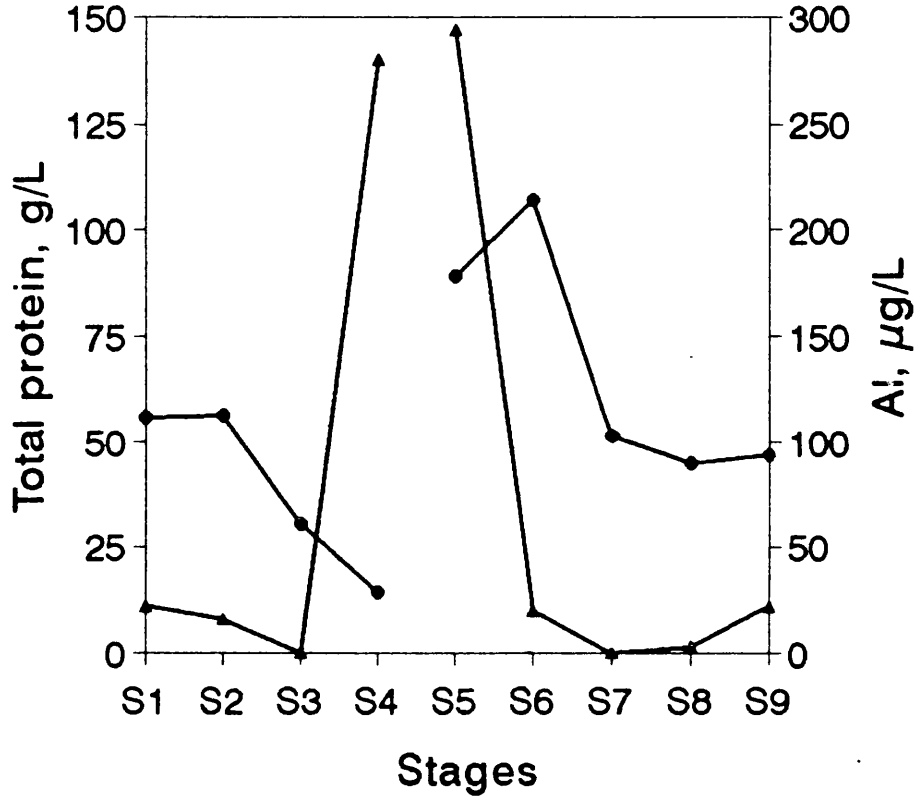

Fig. 2.5 Concentration of Al (triangles) and total protein (circles) in various stages of the production of PPS.

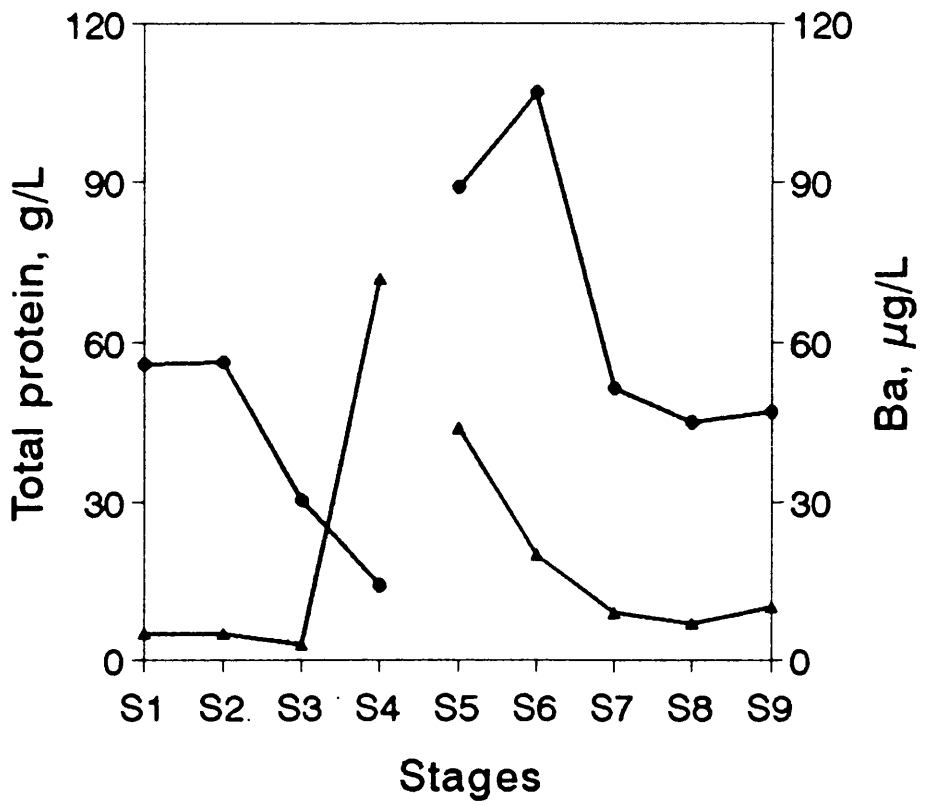

Fig. 2.6 Concentration of Ba (triangles) and total protein (circles) in various stages of the production of PPS.

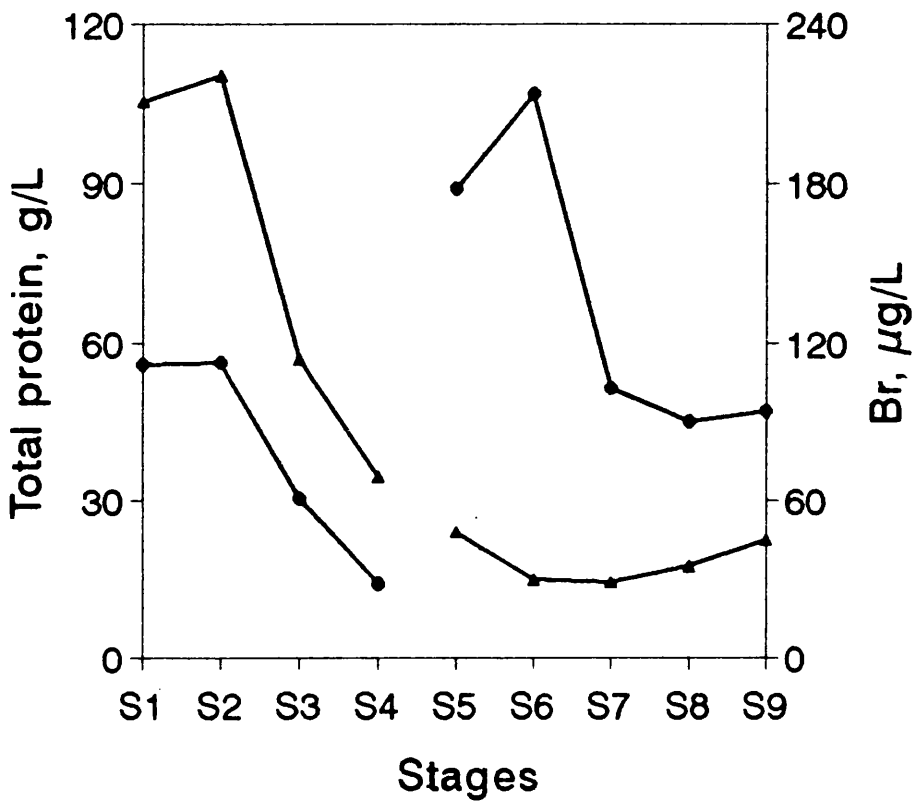

Fig. 2.7 Concentration of Br (triangles) and total protein (circles) in various stages of the production of PPS.

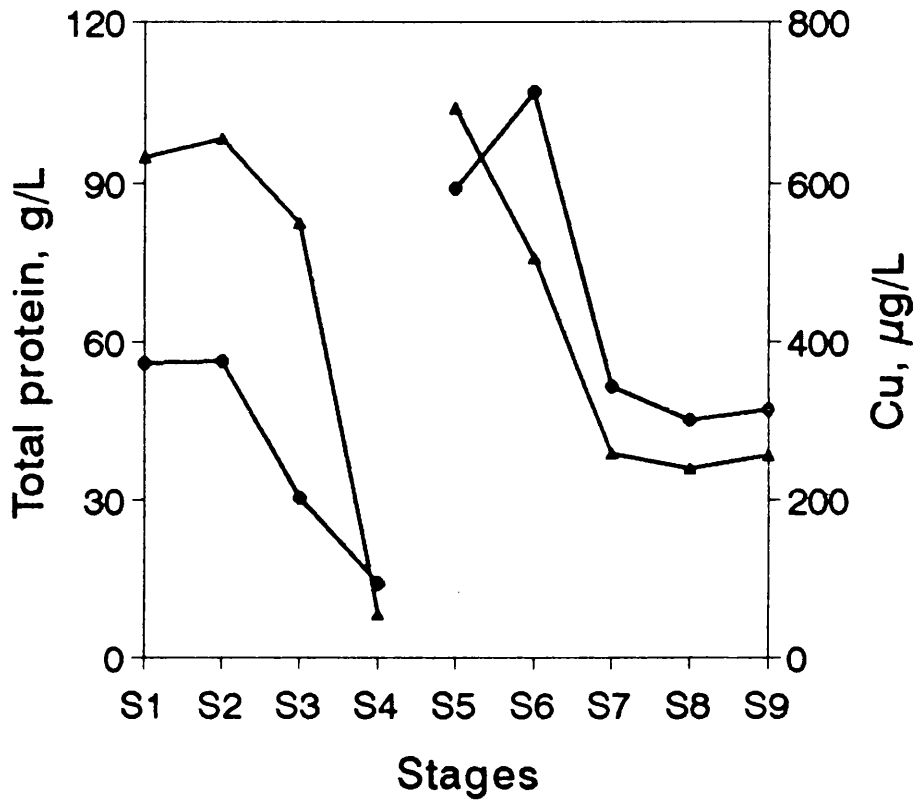

Fig. 2.8 Concentration of Cu (triangles) and total protein (circles) in various stages of the production of PPS.

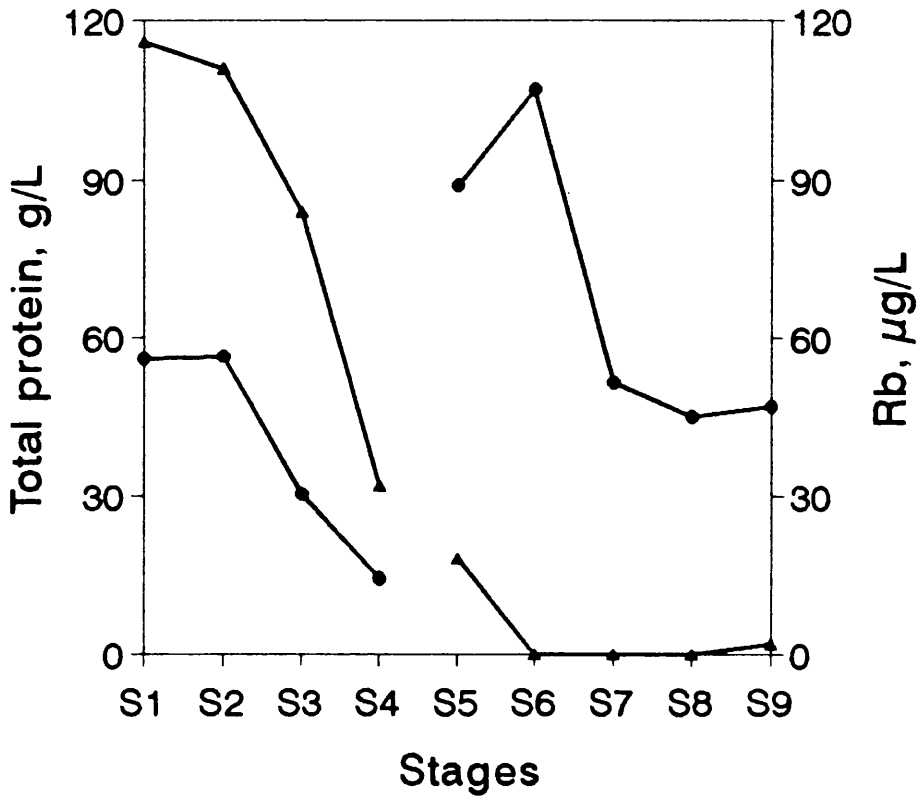

Fig. 2.9 Concentration of Rb (triangles) and total protein (circles) in various stages of the production of PPS.

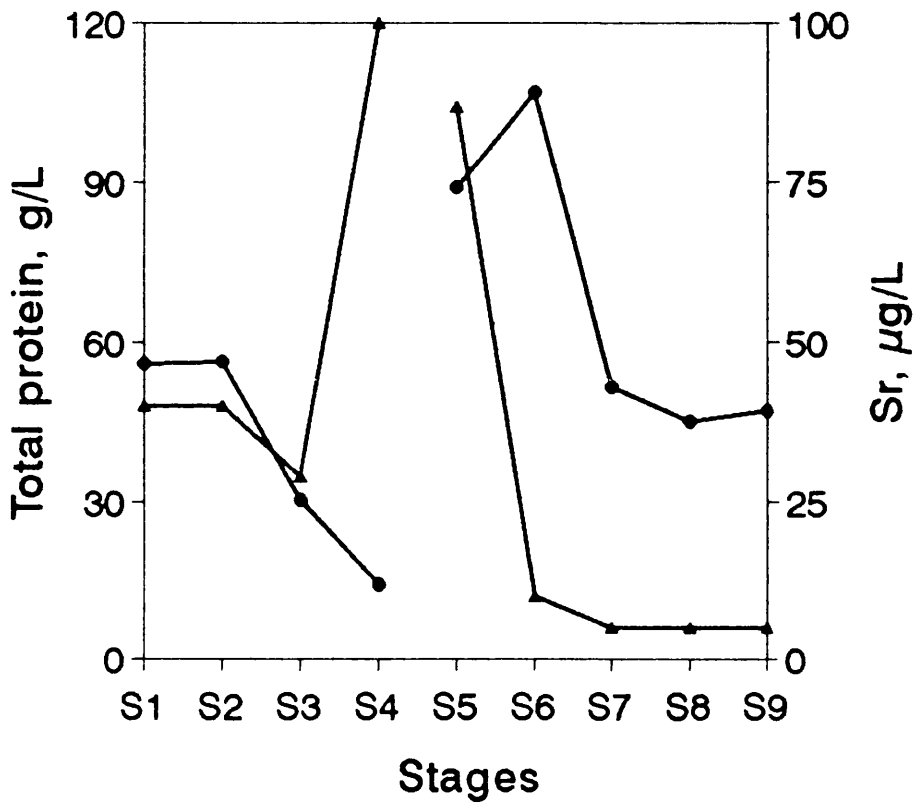

Fig. 2.10 Concentration of Sr (triangles) and total protein (circles) in various stages of the production of PPS.

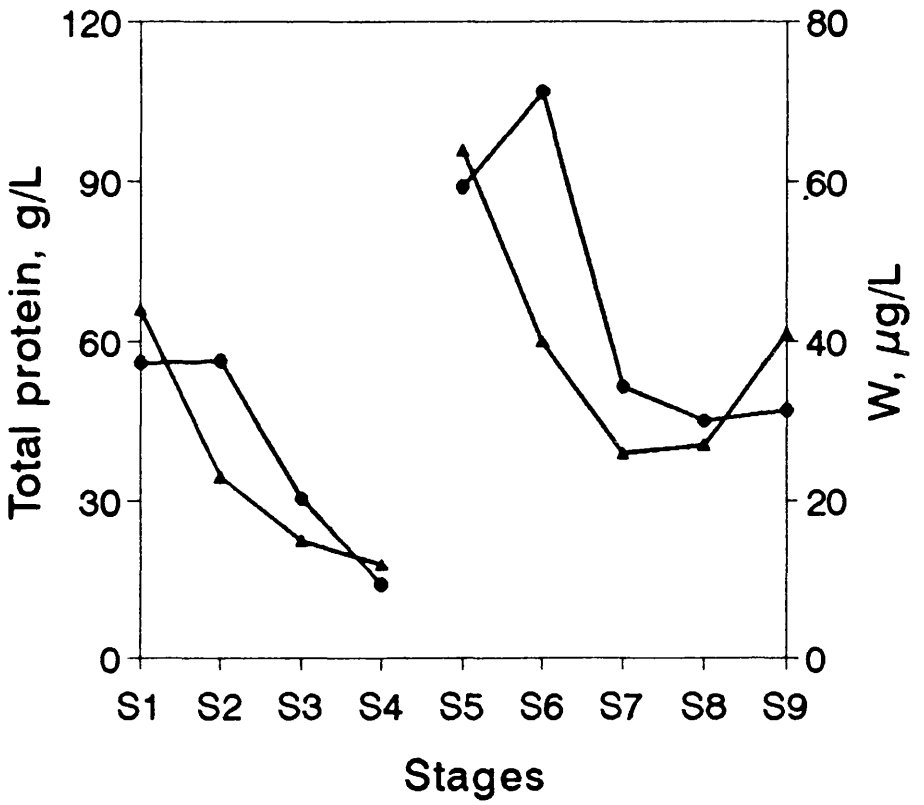

Fig. 2.11 Concentration of W (triangles) and total protein (circles) in various stages of the production of PPS.

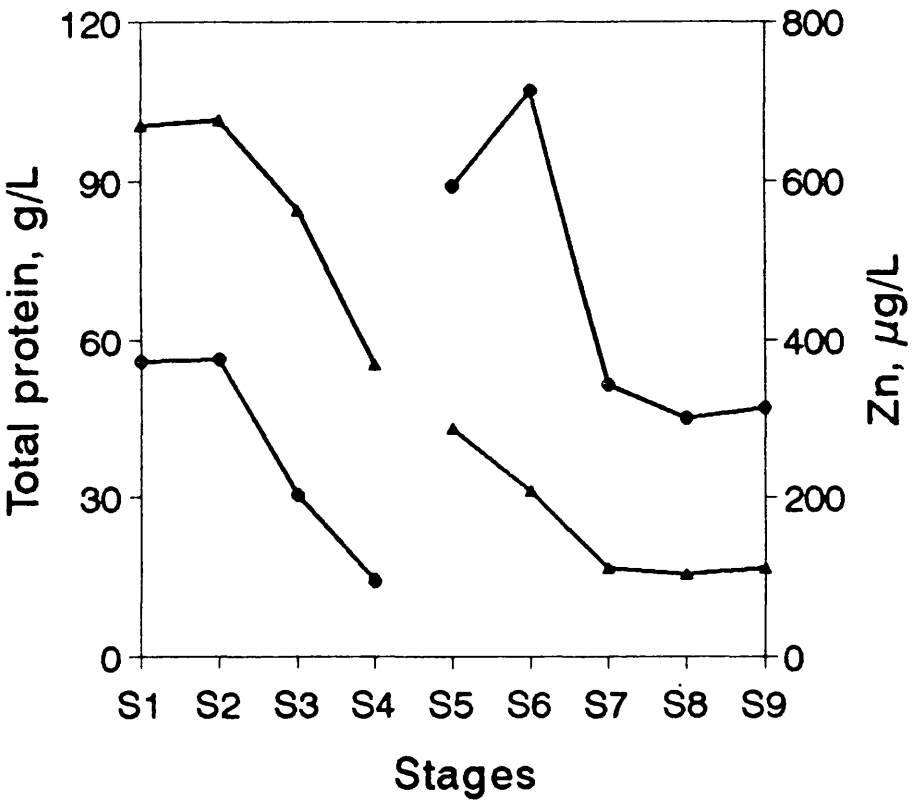

Fig. 2.12 Concentration of Zn (triangles) and total protein (circles) in various stages of the production of PPS.

Fig. 2.4) showed a sharp increase after the buffer addition (S2), then followed the decrease of protein concentration, due to ethanol addition and protein precipitation (S3). After the filtration steps, Ni concentrations were further increased in both the supernatant (S4) and the separated protein fraction containing albumin (S5). The ultrafiltration removed only a small amount of Ni (S6), whereas the rest was tightly bound to albumin (S7, S8). A further increase of Ni concentration occurred in the last step, which included the concentration of proteins from 4.3 to 4.7%.

Other metals showed a different behaviour. Large amounts of Al, Ba and Sr (Table 2.6, Figs. 2.5, 2.6 and 2.10) were absorbed during the filtration steps, but were efficaciously removed by ultrafiltration. The final Sr concentration was actually lower than the initial concentration. Bromine, Rb and Zn (Table 2.6, Figs. 2.7, 2.9 and 2.12) were continuously depleted during the process, whereas Cu (Table 2.6, Fig. 2.8) was only partially lost. Traces of W were present during the whole process but the concentration in the final product did not exceed 40 µg/L.

At present, HAS produced in at least two European countries (UK and Italy) meets desirable standards of quality with regard to Ni concentration. Comparable performances are likely to be obtained in countries who adopt similar procedures, although the risk of contamination should not be

underestimated. For other metals, especially Al, large variability still exists between different manufacturers and batches (Table 2.5).

From the investigation of the production process at the Edinburgh Fractionation Centre, I found that the sources of Ni contamination were the same as described in earlier studies (Maharaj, 1986; Gammelgaard and Sandberg, 1989), but the extent of the contamination was much lower with a Ni content in the final product of only 5.1 µg/L. The filters were found to release considerable amounts of metals, whereas most of the loss of trace elements such as Cu and Zn is likely to be due to the removal of their carrier-proteins. Since the final Zn content in some of the products was very high, leakage from the glass bottles and rubber closure should also be considered as a source of contamination.

2.3 HYPERNICKELAEMIA IN PATIENTS WITH CHRONIC RENAL FAILURE MAINTAINED ON HAEMODIALYSIS

Patients with chronic renal failure (CRF) undergoing regular haemodialysis may experience depletion of essential elements as well as uptake of toxic metals present as contaminants in the dialysis fluids.

The accumulation of Al is a recognised cause of various haemodialysis-related syndromes and the uptake of other toxic elements has also been of concern.

Salvadeo et al. (1979) measured the concentration of 15 elements in the fluid entering and leaving the dialyzer and found a significant reduction in seven potentially toxic metals, including Ni.

Acute Ni intoxication occurred in 23 dialysed patients, who reported symptoms of nausea, vomiting, weakness and palpitations, due to the elution of Ni from a Ni-plated stainless-steel heater into the water supply used to prepare the dialysis fluid (Webster et al., 1980).

Olerud et al. (1984) observed acute allergic reactions to Ni during haemodialysis in a patient with Ni hypersensitivity. The apparent source of Ni was a stainless steel fitting in a bicarbonate delivery system. In 'in vitro' experiments, Olerud et al. (1984) demonstrated that Ni was extracted from the dialysis fluid into the blood. This observation was

consistent with the high affinity of albumin for Ni. After a single haemodialysis cycle, plasma Ni concentration was 89% higher than in the original dialysis fluid.

Drazniowsky et al. (1985) found increased Ni concentrations in 16 patients who were undergoing regular haemodialysis for 1 to 69 months when compared to 71 healthy controls.

Wills et al. (1985) investigated the Al and Ni content of serum and lymphocytes in long-term haemodialysed patients. Serum Ni concentrations were higher in patients than controls; in contrast, there was no significant difference in the Ni concentration of lymphocytes between the two groups. Wills et al. (1985) concluded that Ni was not retained in tissue. However, due to the very low amounts present, contamination or analytical difficulties may have obscured such a finding.

Hopfer et al. (1985, 1989) carried out an extensive study of Ni concentrations in serum of patients with CRF attending different dialysis facilities. Hypernickelaemia was a consistent finding in all the haemodialysed patients. Only one of 7 subjects with CRF not undergoing dialysis had elevated serum Ni compared with controls. Hopfer et al. (1985) found increased serum and whole blood Ni concentrations after a single dialysis cycle and observed the reduction of serum Ni concentrations in patients when water of improved purity was used to

make up the dialysis fluid.

Nixon et al (1989), using improved methodology and strict contamination control, observed values of Ni in serum ranging from 1.5 to 18.9 µg/L in patients maintained on dialysis, whereas the average serum Ni concentration in their controls was 0.14 ± 0.09 µg/L.

Hosokawa et al. (1987a) reported lower Ni concentrations in subjects undergoing dialysis, compared with an average value of 5.6 ± 0.8 µg/L in controls, and a significant correlation between dialysis anaemia and serum Ni concentrations (1987b).

These results suggest some positive evidence of Ni transport between the dialysis fluid and blood, even against a concentration gradient. The results of these investigations are contradictory and no correlation was found between Ni concentrations and length of therapy, age or sex of the subjects. Little is known about the mechanisms of Ni uptake during dialysis and its metabolic fate in patients with impaired renal function.

The observation that most of the patients maintained on haemodialysis have hypernickelaemia suggests that Ni is either absorbed during dialysis or not completely removed from the body by this treatment and may accumulate in bone and tissues. Hopfer et al. (1985) highlighted the similarity between a number of disorders observed in patients undergoing long-term haemodialysis and effects observed in rodents after parenteral

administration of $NiCl_2$, such as lipid peroxidation (Giardini et al., 1984), impaired cellular and humoral immunity (Graham et al., 1978; Donnelly et al., 1983; Smialowicz et al., 1984), and hyperprolactinaemia (Clemons and Garcia, 1981; Mastrogiacomo et al., 1984).

The clinical significance of Ni in haemodialysis may have been confounded by factors such as the limited number of subjects studied, the high risk of contamination and the difficulties of Ni analysis at low concentrations.

In recent years, efforts have been made to meet requirements of purity for dialysis fluids and concentrates, in agreement with the EEC resolution on protection of haemodialysed patients from Al toxicity (Council of the European Communities, 1986). Therefore, risk of contamination from other metals may also be reduced and the extent and the causes of hypernickelaemia in haemodialysed patients may need to be reconsidered.

Nowadays, improved analytical methodologies and means of contamination control are available to a larger number of laboratories. Nixon et al. (1989) needed to carry out sample preparation and analysis of serum Ni in a 'class 100' clean environment, to accurately determine reference values in a normal population. However, the analysis of the higher concentrations of Ni expected in haemodialysed patients could be

carried out under less strict conditions, provided that there is careful control of contamination during sample collection and analysis.

I assessed the performance of a method for serum Ni analysis, based on graphite furnace atomic absorption spectrometry with deuterium-arc background correction (D2-GFAAS). This instrumentation is available to a larger number of laboratories than Zeeman corrected GFAAS (Z-GFAAS), used in the most recent methods (Sunderman et al., 1984; Andersen et al., 1986; Nixon et al., 1989).

I also measured the concentrations of serum Ni in a group of 25 patients undergoing regular haemodialysis and investigated the effect of a single dialysis cycle on serum Ni concentrations.

2.3.1 Experimental

Subjects: Blood samples were obtained from 25 patients with end-stage chronic renal failure (18 men and 7 women, age 22 to 78 years, mean ± s.d.: 53 ± 17 years), who had been treated by haemodialysis three times a week at the Renal Unit of Glasgow Royal Infirmary for an average of 42 ± 59 months (range >1-290 months).

Dialysis was performed with equipment from Gambro Dialysatoren, GmbH & Co, Munich, Germany and Fresenius AG, Munich, Germany,

using capillary flow dialysers with Cuprophan membranes (Baxter Healthcare Ltd, England, CF ST15, membrane surface 0.9 m^2, and CF ST23, membrane surface 1.25 m^2) or cellulose acetate hollow fibre dialysers (Baxter, CA150, membrane surface 1.5 m^2). Conventional electrolyte concentrate solutions, manufactured by Gambro and Fresenius, respectively, were diluted 1+34 with water purified by reverse osmosis.

Serum Ni concentrations were also measured in a control group of 8 women with normal renal function, age 29 to 73 years (mean ± s.d.: 48 ± 19 years), who were receiving total parenteral nutrition (TPN) and being monitored for essential trace element status.

Contamination control: Due to the ubiquitous presence of Ni in the environment and the 'scavenging' properties of human albumin, strict precautions were taken to avoid contamination of samples during the pre-analytical and analytical phases. Ultrapure HNO_3, 65%, ('Aristar', BDH), was further purified by subboiling in PTFE bottles. Ultrapure water was obtained by a four-stage purification using ion-exchange (Elgastat UHP, Elga, High Wycombe, U.K.). All plastic-ware (i.e. tubes, Pasteurs, AAS cups) were soaked overnight in 20% HNO_3, rinsed thoroughly six-times with ultrapure water, dried in a laminar flow hood and stored in clean

plastic bags until use. Pipette tips were rinsed three times with 20% HNO_3 and ultrapure water before use. Manipulation of samples was carried out in a laminar flow hood.

Blood collection: Blood samples were obtained before dialysis directly from the intra-arterial cannula. To avoid contamination from needles, the first 10 ml of blood were collected for routine analyses, then another 10 ml aliquot was withdrawn in a plastic tube, allowed to clot for at least an hour and centrifuged at 2500 rpm for 10 min. Serum was transferred into a clean 5 ml plastic tube using a polypropylene pipette and stored at -20 °C.

Post-dialysis samples were obtained with the same procedure from 12 patients (9 men, 3 women, age 53 ± 21 years, range 23-78, average time on dialysis 37 ± 20 months, range 8-67 months).

Blood samples from the TPN subjects were taken using a plastic I.V. cannula.

Nickel analysis: Ni determinations were carried out by GFAAS. Instrumentation and operative conditions were as described for HAS analysis. The graphite furnace programme is reported in Table 2.7. The injection volume was 50 µl.

Table 2.7 Furnace programme for the determination of Ni in serum by GFAAS

STEP	1	2	3	4	5	6
TEMP, °C	100	150	200	1200	2600	2700
RAMP TIME, s	1	50	30	80	0	1
HOLD TIME, s	1	5	5	50	4	3
READ					on	
GAS FLOW, ML/MIN	300	300	300	300*	0	300

* Gas flow =0 for the last 5 s in this step

Working standard solutions containing 0, 2.5, 5, 10 and 20 µg/L Ni were prepared from a Ni stock solution, 1 g/L, ('Spectrosol', BDH), in HNO_3 1% v/v.

Aqueous standards and serum samples were diluted 1+1 with a diluent solution containing 1% v/v HNO_3 and 0.25% v/v Triton X-100. All standards and serum samples were analysed in duplicate. Ni concentrations in the samples were obtained by comparison with a calibration curve obtained from the absorbance of the aqueous standard solutions.

Additional analysis: Serum albumin was determined by the bromocresol green method with an Olympus automatic analyser.

2.3.2 Results and discussion

Analytical performances of the method: The plot of absorbance versus Ni concentration was linear within the range 0 to 20 µg/L. Using a prolonged ashing time, the background signal observed during the analysis of serum samples was maintained below 0.150 A·s and could be corrected by the deuterium background system. A long ramp time was found necessary to avoid the build-up of carbonaceous

residues. With these conditions, matrix interferences were reduced and a calibration graph obtained with aqueous Ni solutions could be used for quantitation, but only when absorbance was measured as peak area. The plots of peak area versus Ni concentration obtained with either aqueous or serum-based standard solutions were parallel (regression lines equations: aqueous solutions, $y=0.2\cdot10^{-3} + 7.78\cdot10^{-3}x$, $r^2=0.998$; serum-based standards, $y=24.9\cdot10^{-3} + 7.82\cdot10^{-3}x$, $r^2=1.000$). No significant difference was observed between the concentrations of 38 serum samples, within the range 1.5-4.5 µg/L, determined using both aqueous and serum-based calibration standards (paired data t-test, average difference -0.06 ± 0.13 µg/L). The peak height signal measured for equal amounts of Ni was higher for serum-based standards than for aqueous solutions (regression lines equations: aqueous solutions, $y=1.3\cdot10^{-3} + 6.30\cdot10^{-3}x$, $r^2=1.000$; serum-based standards, $y=22.7\cdot10^{-3} + 6.67\cdot10^{-3}x$, $r^2=0.999$).

The detection limit, (three times the standard deviation of 10 replicate measurements of the blank) was 0.15 µg/L, equivalent to 0.3 µg/L in the undiluted sample. The characteristic mass (mass of analyte in pg that yields a signal of 0.0044 A·s) was 14 pg. Within-day precision was 5.6% and between-day precision was 7.5%. The average recovery of known amounts of Ni (2.5, 5.0 and 10.0 µg/L) added to a serum sample was 101 ± 4.8% (n=9).

The analysis of Seronorm Trace Element Control Serum (Nycomed AS Diagnostics, Oslo, Norway, 3.2 µg/L) and RM 8419, Bovine Serum (National Institute of Standards and Technology, Gaithersburg, MT, USA, 1.8 µg/L) by this method gave average values of 3.21 ± 0.17 µg/L (n=10) and 0.46 ± 0.05 µg/L (n=4), respectively. A similar discrepancy with the certified value of the RM 8419 has been recently reported by two other researchers (Andersen et al., 1986; Nixon et al., 1989), who suggested that the recommended value of 1.8 µg/L may be in error.

This method compares well with Z-GFAAS procedures, in terms of characteristic mass, precision and accuracy (Table 2.8). It shows better precision and detection limit than those previously reported for D2-GFAAS using older instrumentation (Drazniowsky et al., 1985), although their value for the characteristic mass was much lower. On the other hand, the detection limit is higher than those reported for procedures that apply Zeeman correction. Attempts to improve the sensitivity using a multiple injection failed, because of the unmanageable increase in background signal.

Therefore, the normal concentrations of Ni in serum of unexposed subjects cannot be determined by this method. However, the measurement of such low concentrations requires specialised facilities for the control of contamination, as described by Nixon et al. (1989), and is

Table 2.8 Analytical performances of this method in comparison with other procedures.

Method	Sunderman et al 1984	Drazniowsky et al. 1985	Andersen et al. 1986	Nixon et al. 1989	This method 1992
Background correction	Zeeman	Deuterium	Zeeman	Zeeman	Deuterium
Sample pre-treatment	Deproteinisation	Dilution (1+1)	Dilution (1+1)	Dilution (3+1)	Dilution (1+1)
Injection	Single	Single	Single	Multiple	Single
Injection volume	50	50	50	100	50
Calibration	Aqueous standards	Serum-based standards	Serum-based standards	Serum-based standards	Aqueous standards
Performance					
Detection limit, µg/L	0.05	0.9	0.09	0.06	0.15
Characteristic mass*, pg	27.9	6.3	13	11	14
Precision:					
Within-day, RSD%	3.8	6.3	-	3.2	5.6
Between-day, RSD%	8.1	34.9	-	-	7.5
Accuracy:					
SERONORM (3.2 µg/L)	-	-	2.93 ± 0.34	3.30 ± 0.23	3.21± 0.17
SRM 8419 (1.8 µg/L)	-	-	0.48 ± 0.04	0.50	0.46 ± 0.05
Average recovery (%)	97 ± 2.7	99 ± 6	-	-	101.5 ± 4.8
Range	94-103	86-118	-	-	92-107

* Mass of Ni which gives a signal of 0.0044 A · s

confined to a limited number of research centres.

Our method, using widely available instrumentation, can be applied by less specialised laboratories to monitor Ni exposure, in clinical and occupational toxicology.

Nickel concentrations in serum of haemodialysed subjects and controls: The concentrations of Ni measured in serum of 25 patients maintained on haemodialysis are reported in Table 2.9. The average value was 2.4 ± 0.9 µg/L (range 0.7-4.0 µg/L). In comparison, serum Ni concentrations in 8 women who were receiving TPN, but had normal renal function, ranged between 0.3 and 0.9 µg/L (Table 2.10) and the average value was 0.5 ± 0.2 µg/L. The distribution of the observed values is shown in Fig. 2.13.

Although the control group could be theoretically exposed to Ni present as contaminant in nutrient solutions, Berner et al. (1989) have observed that the daily intake of Ni received by patients maintained on TPN is comparable with the amounts reported to be absorbed through the gastrointestinal tract in healthy subjects. This amount is rapidly eliminated by the kidney, provided that the renal function is not affected. The values observed for serum Ni concentrations in the TPN subjects are comparable with those reported for healthy volunteers (Table 2.11), except Nixon et al. (1989). However, because Ni concentrations lower than 0.3 µg/L are not

Table 2.9 Serum Ni concentrations in haemodialysed patients.

Subject N.	Sex	Age (yrs)	Diagnosis	Months on dialysis	Serum Ni µg/L
#1	M	78	CRF	48	1.9
#2	M	61	CRF	1	2.6
#3	M	66	CRF-Hypoplastic kidneys	45	1.8
#4	M	22	CRF	26	3.8
#5	F	47	CRF	88	2.8
#6	M	46	CRF	1	0.7
#7	F	61	Mesangiocapillary GN	1	3.6
#8	M	71	CRF	7	1.1
#9	F	71	CRF	73	2.4
#10	F	47	CRF	<1	2.0
#11	M	41	CRF	66	2.9
#12	M	55	CRF	27	2.2
#13	M	49	End-stage CRF	290	1.9
#14	M	33	CRF	1	2.8
#15	M	58	CRF	83	2.7
#16	M	62	Crescentis GN	9	1.1
#17	M	39	CRF	49	2.6
#18	M	38	CRF	67	2.0
#19	F	74	CRF	17	1.0
#20	M	77	CRF	36	2.6
#21	M	68	CRF	3	4.0
#22	F	39	CRF	37	3.7
#23	F	23	End-stage RF	23	1.5
#24	M	24	End-stage RF- small kidneys	40	3.7
#25	M	72	CRF	8	2.1
MEAN		53		42	2.4
S.D.		17		57	0.9
MEDIAN		55		27	2.4

CRF=chronic renal failure; GN=glomerulonephritis

Table 2.10 Serum Ni concentrations in patients receiving TPN

Subject N.	Sex	Age (yrs)	Serum Ni, µg/L
#1	F	73	0.3
#2	F	69	0.5
#3	F	29	0.9
#4	F	29	0.3
#5	F	30	0.4
#6	F	68	0.5
#7	F	31	0.7
#8	F	56	0.5
MEAN		48	0.5
S.D.		19	0.2

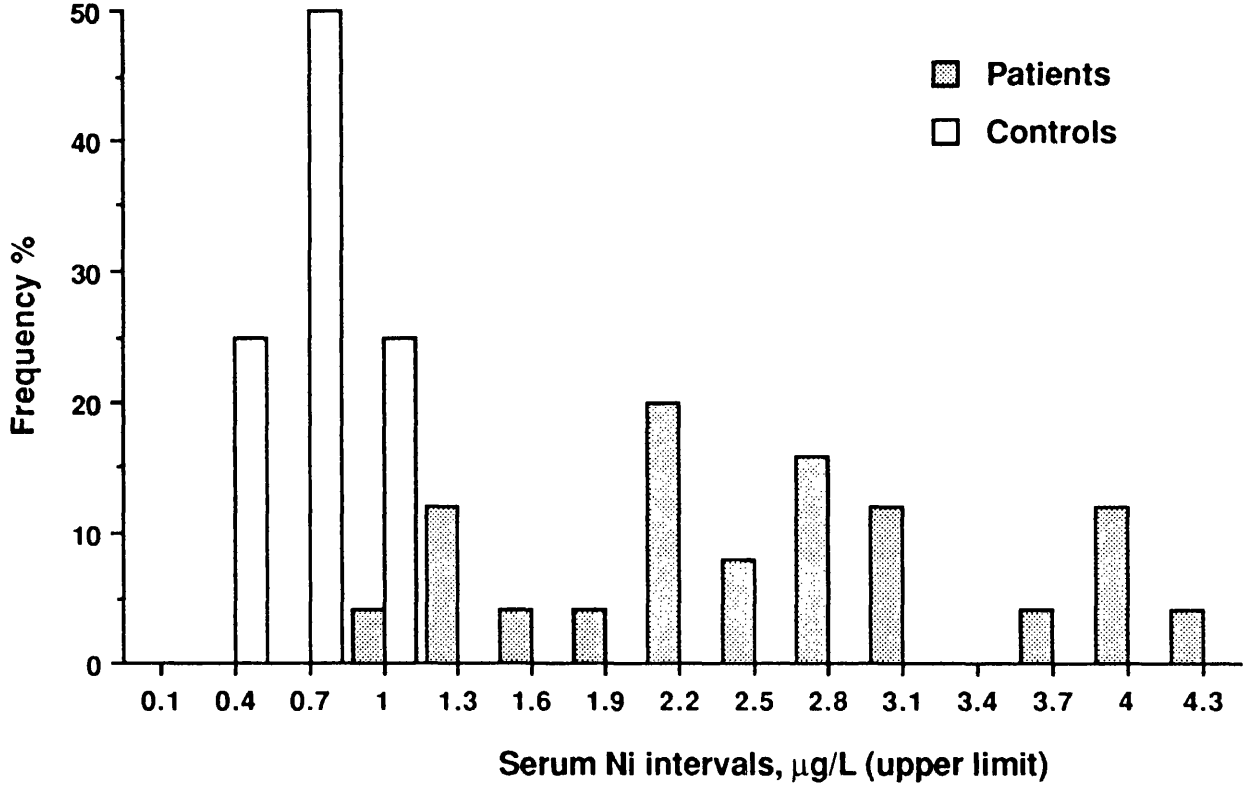

Fig. 2.13 Frequency distribution of serum Ni concentrations in controls and haemodialysed patients.

Table 2.11 Serum Ni concentrations (mean ± s.d., µg/L) in haemodialysed subjects and controls in earlier studies and this investigation.

Reference	Controls	N	Patients	N
Hopfer et al., 1985	0.3 ± 0.2	30	5.4 ± 2.1	65
Drazniowsky et al., 1985	1.0 (0.6-1.4)*	71	7.4(6.0-9.1)*	16
Wills et al., 1985	0.44 ± 0.18	18	3.71 ± 1.54	28
Hosokawa et al., 1987	5.6 ± 0.8	30	2.2 ± 0.3	30
Hopfer et al., 1989	0.6 ± 0.3	22	7.0 ± 2.4	30
Nixon et al., 1989	0.14 ± 0.09	38	6.38 ± 0.18	40
This work, 1993	0.5 ± 0.2	8	2.4 ± 0.9	25

* median (lower-upper quartile)

detectable with this method, the average value of Ni concentrations in the control group may be overestimated.

Serum Ni concentration does not appear to correlate with the length of dialysis. The average serum Ni concentrations observed in this study for subgroups of patients maintained on dialysis for: less than 10 months (n=9); 10 to 49 months (n=10); 50 to 88 months (n=5) and 290 months (n=1) were 2.2 ± 1.1 µg/L; 2.5 ± 0.9 µg/L; 2.6 ± 0.3 µg/L and 1.9 µg/L, respectively. Other researchers (Hopfer et al., 1985; Nixon et al., 1989) reported that, in their groups of haemodialysed patients, only those who had started the dialysis treatment less than 13 months ago had lower serum Ni concentrations compared with the others.

The serum Ni concentrations observed for haemodialysed subjects in this study are lower than those reported by other researchers (Drazniowsky et al., 1985; Wills et al., 1985; Hopfer et al., 1985; Hopfer et al., 1989; Nixon et al., 1989) (Table 2.11). This may reflect the improvement of the purity of water and electrolyte concentrate solutions now used. The Ni concentration in samples of dialysis fluid, collected just before and after the dialyser from 5 dialysis sets, was lower than the detection limit of our method (0.45 µg/L for samples diluted 1+2).

Hopfer et al. (1989) observed a significant reduction in serum Ni concentrations of a group of haemodialysed patients after six months

since the reduction of the Ni content of the dialysis fluid (from 0.82 μg/L to 0.53 μg/L) due to the introduction of a new reverse osmosis system for the purification of the water.

The analysis of serum samples, obtained in the same day from 12 patients before and after dialysis (Tables 2.12, 2.13), yielded average Ni concentrations of 2.2 ± 0.9 μg/L and 3.0 ± 0.9 μg/L, respectively, ($p<0.01$, paired-data t-test). The average increase in serum Ni concentrations after a single treatment of dialysis was only slightly lower than those reported by Hopfer et al. (1985; 1989) (Table 2.12), despite the lower serum Ni concentrations observed in our group of subjects. On the contrary, the increment of serum albumin in post-dialysis specimens (pre-dialysis value: 41 ± 3.5 g/L; post-dialysis value: 45 ± 5.6 g/L; average difference: 4 ± 5 g/L), due to haemoconcentration, was comparable with the values of 10% and 8% observed by Hopfer et al. (1985; 1989) (Table 2.13).

Although statistically significant, the Ni increase in our group of subjects was very variable and did not correlate with the increment of serum albumin or with pre-dialysis serum Ni values. In addition, two patients had reduced serum Ni concentrations after dialysis, despite the rise of albumin concentrations (Table 2.13). A large variability may also be observed in the results reported by Hopfer et al.(1985; 1989) (Table 2.12). When individual post - dialysis serum Ni concentrations were corrected for

Table 2.12 Serum Ni concentrations (mean ± s.d., µg/L) in haemodialysed subjects before and after a single dialysis treatment reported in earlier studies and in this investigation.

Reference	Pre-dialysis	Post-dialysis	Difference	N	p
Hopfer et al., 1985	6.2 ± 1.8	7.2 ± 2.2	1.0 ± 1.1	40	<0.01
	3.9 ± 2.0	5.2 ± 2.5	1.3 ± 1.0	9	<0.01
	3.0 ± 1.3	3.7 ± 1.3	0.7 ± 0.3	10	<0.01
Hosokawa et al., 1987	2.2 ± 0.3	2.5 ± 0.5	0.3 ±0.3	30	<0.01
Hopfer et al., 1989	7.0 ± 2.4	8.5 ± 2.8	1.5 ±1.3	30	<0.01
This work, 1993	2.2 ± 0.9	3.0 ± 0.9	0.8 ±0.9	12	<0.01

Table 2.13 Serum Ni and albumin concentrations observed in this study in 12 subjects before (Pre-d.) and after (Post-d.) a single dialysis treatment. Comparison with post-dialysis serum Ni adjusted for the variation of blood volume using individual albumin values.

Subject	Ni (µg/L)			Albumin (g/L)			Adjusted	
Code	Pre-d.	Post-d.	Diff.[a]	Pre-d.	Post-d.	Diff.[a]	Post-d. Ni (µg/L)	Diff.[a]
#1	1.9	3.2	1.3	34	38	4	2.9	1.0
#3	1.8	2.6	0.8	40	43	3	2.4	0.6
#11	2.9	3.5	0.6	41	39	-2	3.7	0.8
#16	1.1	3.6	2.5	38	43	5	3.2	2.1
#17	2.6	2.7	0.1	42	41	-1	2.8	0.2
#18	2.0	3.4	1.4	42	45	3	3.2	1.2
#19	1.0	2.3	1.3	40	46	6	2.0	1.0
#20	2.6	3.5	0.9	38	43	5	3.1	0.5
#22	3.7	4.7	1.0	44	55	11	3.8	0.1
#23	1.5	1.7	0.2	48	42	-6	1.9	0.4
#24	3.7	3.1	-0.6	43	51	8	2.6	-1.1
#25	2.1	1.7	-0.4	42	54	12	1.3	-0.8
Mean	2.2	3.0	0.8	41.0	45.0	4.0	2.7	0.5
sd	0.9	0.8	0.8	3.3	5.3	5.0	0.7	0.8
p[b]			<0.01			< 0.025		n.s.

[a] difference between pre- and post-dialysis values

[b] Paired-data Student t test

haemoconcentration using the serum albumin values, the average concentration was 2.7 ± 0.7 µg/L and the increase versus pre-dialysis values (mean ± s.d.: 0.5 ± 0.8 µg/L) was lower and not significant by the paired-data t-test (Table 2.13).

Despite of the improvement of the purity of dialysis fluids, hypernickelaemia, although moderate, still occurred in our group of haemodialysed patients. The exchange of Ni 'in vivo' during dialysis appears to be variable and depending on factors that have not yet been completely clarified.

2.4 CONCLUSIONS

The clinical toxicity of Ni has been considered with regard to two well known situations in which adverse effects due to Ni contamination may occur: intravenous administration of human albumin solutions and haemodialysis. The results of this study showed that the concentration of Ni in human albumin solutions has been consistently reduced during the past few years and is now largely within limits considered safe. At the same time, the serum Ni concentrations of a group of haemodialysed patients appear lower than earlier reports suggest. This reduction can be

attributed to improved purity of the dialysis fluids. However, serum Ni concentrations in haemodialysed patients remain elevated in comparison with healthy subjects, do not correlate with the length of time since dialysis started and post-dialysis values did not differ significantly from pre-dialysis values after correction for haemoconcentration. Although patients do not suffer from known adverse effects due to Ni toxicity, the reason why patients maintained on dialysis have hypernickelaemia remains unclear. The high concentration of contamination of dialysis fluids noted earlier may have obscured factors other than dialysis that may also affect serum Ni concentrations in subjects with impaired renal function. A better understanding of Ni metabolism may prove helpful in identifying some of these factors.

CHAPTER 3

DETERMINATION OF NI ISOTOPES BY INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

3.1 INTRODUCTION

Inductively coupled plasma mass spectrometry (ICP-MS) is a powerful technique for the determination of metals, allowing multielemental analyses, improved sensitivity for a number of elements and the determination of the isotopic composition of the sample.

Instrumentation consists of an inductively coupled plasma torch, placed horizontally and interfaced with a quadrupole mass analyser, by means of especially designed metallic cones. A system of ion lenses is placed behind the cones to form an ion beam, which enters the mass spectrometer. Different ions are separated according to their mass/charge ratio and can be unequivocally identified. The region between the two cones, the ion lenses system and the mass spectrometer are evacuated to a low pressure by cryogenic and mechanical pumps. A computerised

system provides automatic control of the various functions of the instrument, data acquisition and handling. Samples are introduced to the torch by pneumatic or ultrasonic nebulisation, generally after a dissolution and/or dilution step.

The mass spectra of the elements are considerably simpler than their optical emission spectra; however, interferences may arise from overlapping of naturally abundant isotopes of different elements and polyatomic species formed at high temperature from the carrier gas and ions present in the sample. In complex matrices these interferences can drastically increase the detection limits and reduce the number of isotopes that can actually be used for analytical purposes.

The availability of a relatively simple and rapid technique to obtain isotopic information provides the opportunity to apply stable isotopes as tracers for the study of mineral metabolism in humans and for accurate analytical methods based on isotopic dilution (ID).

Isotope dilution analysis consists of the measurement of isotopic ratios in two aliquots of a sample, one of which has been spiked with a known amount of an isotope of the element to be determined. The concentration of the element in the sample can be calculated from the measured isotopic ratios, the amount of the spike and the weight or volume of the sample. The uncertainties of the measure of these quantities are the only

sources of error that can affect the results, since calibration is not needed. Losses during the pretreatment of the sample do not modify the isotopic ratios. This procedure offers high precision and accuracy and has already been applied using thermal ionisation mass spectrometry and spark source mass spectrometry for the determination of isotopic ratios. These techniques are available to a limited number of laboratories, are more time consuming and require more skilful instrument operation and complicated sample preparation than ICP-MS.

The establishment of methods of high accuracy based on ID-ICP-MS for the determination of trace elements in different biological matrices is important for investigations of the roles of trace metals in human health and disease, as well as the certification of reference materials and the development of reference and routine analytical methods.

Nickel, with five naturally abundant stable isotopes, is an ideal element for such experiments. The need for the development of a definitive method, based on ID-MS, for the determination of Ni in biological materials of human origin, had already been recognised in 1980 by the International Union of Pure and Applied Chemistry (IUPAC, 1980b). Since then, the only reported attempt to meet this need was a procedure based on isotopic dilution-gas chromatography-mass spectrometry, (Aggarwal et al., 1989a, 1989b). The Authors determined Ni isotopes in a freeze-dried

urine reference material (US National Institute of Standards and Technology, Standard Reference Material 2670, recommended value 70 µg/L), after the development of thermally stable, volatile Ni complexes with lithium bis(trifluoroethyl)dithiocarbamate.

Inductively coupled plasma mass spectrometry has been applied to the determination of Ni in marine biological reference materials such as lobster hepatopancreas (Ridout et al., 1988), dogfish liver and muscle tissue (Beauchemin et al., 1988a), cod liver and shellfish tissue (Beauchemin et al., 1988b), protein plasma solutions (Diver et al, 1988; Lyon et al., 1988a), urine and blood serum (Lyon et al., 1988a; Vaughan and Templeton, 1990; Xu et al., 1993). In these studies, the determination of all Ni isotopes was affected by a number of isobaric and polyatomic interferences (Table 3.1), arising from ions commonly present at much higher concentration than Ni in biological samples. In particular, the determination of the most abundant Ni isotope (^{58}Ni) is often precluded by the overlapping with a minor Fe isotope. Therefore, most of the determinations of Ni by ICP-MS were carried out at mass 60, using the second most abundant Ni isotope, which was less affected by interferences.

In addition, other problems were identified.

Table 3.1 Possible isobaric and polyatomic interferences affecting the determination of Ni isotopes in biological materials by means of ICP-MS. (% Natural abundance of isotopes).

ISOTOPE MASS	POSSIBLE ISOBARIC AND POLYATOMIC INTERFERENCES
^{58}Ni (67.76%)	^{58}Fe (0.31%), ^{42}CaO, NaCl
^{60}Ni (26.16%)	$^{43}CaOH$, ^{42}CaO
^{61}Ni (1.25%)	$^{44}CaOH$
^{62}Ni (3.66%)	^{46}CaO, Na_2O, NaK
^{64}Ni (1.14%)	^{64}Zn (43.9%), $^{32}SO_2$, $^{32}S_2$, ^{46}CaO

Ridout et al. (1988) observed lower Ni values than expected in digested lobster tissue and attributed this discrepancy to signal suppression from high total dissolved solid content.

Beauchemin et al. (1988a, 1988b) reported significant interferences, due to Ca oxides and hydroxides, at mass 60 and 61, with the result that neither the 61/60 nor the 62/60 isotope pairs could be used for the determination of Ni by the ID technique in marine reference materials. However, the results provided by the standard addition method on ^{58}Ni (Beauchemin et al, 1988a) or external calibration using ^{60}Ni (Beauchemin et al., 1988b) were in good agreement with those obtained in interlaboratory comparisons with various other techniques.

Diver et al. (1988) and Lyon et al. (1988a) observed reasonable agreement between the values obtained by ICP-MS using ^{60}Ni and those provided by GFAAS and ICP-AES in freeze-dried plasma protein solutions with high Ni content (>0.4 mg/L), whereas the results obtained for the analysis of all the elements of the first transition row, including Ni, in commercial freeze-dried control urine were severely degraded by polyatomic interferences (Lyon et al., 1988a).

Besides interferences from Ca oxides and hydroxides at mass 60 and 61, Lyon and Fell (1990) identified as Na_2O a peak at mass 62 observed in diluted serum samples. Vaughan and Templeton (1990) reported

spectral overlaps, attributed to the species NaK and Na_2O, with ^{62}Ni in samples of urine. These Authors used a mathematical treatment (principal component analysis, PCA) to separate the information from the analyte and the overlapping species and were able to determine Ni in a freeze-dried urine reference material (NIST SRM 2670), after a 40% dilution with water. The major problem encountered using mathematical correction of interferences was the unpredictable variability of the species formed, which was not linear with time and also depended on the number and salt content of the analysed samples. Therefore, reference solutions of Ca and Na had to be run frequently to compensate for the drift, the most recent data being used for calculations. In a later paper, Xu, Stuhne-Sekalec and Templeton (1993) applied PCA to the determination of Ni in digested serum, but preferred to reduce the Ca content in urine samples by precipitation as Ca oxalate, since mathematical treatment was not robust enough for routine analysis of Ni concentrations in urine (1-5 µg/L Ni).

The determination of Ni isotopes in other human biological materials, such as red cells and faeces, which are important for metabolic studies, have not yet been reported.

I investigated the use of separation techniques to eliminate the ions causing interferences on the determination of Ni isotopes in biological materials. Using size-exclusion chromatography (SEC), I reduced the

sodium content of human albumin solutions and established an ID-MS procedure for Ni analysis. Since SEC was not suitable for other biological materials and lower Ni concentrations, I investigated a more efficient procedure of purification, based on complex formation and solvent extraction. This method of separation had already been successfully used as part of the reference method proposed in 1980 by the IUPAC Toxicology Committee for the analysis of Ni in serum and urine by GFAAS (IUPAC, 1980b). I applied the same procedure, modified to allow for higher sample volume and standardisation with ID, to the determination of Ni isotopes by ICP-MS. Preliminary results were obtained on samples of human plasma, red cells, urine and faeces, spiked with ^{62}Ni and ^{61}Ni, to assess the feasibility of an accurate study of Ni absorption, distribution and excretion in man, using ^{62}Ni as a metabolic tracer and ^{61}Ni for ID.

3. 2 SIZE-EXCLUSION CHROMATOGRAPHY FOR THE REMOVAL OF INTERFERENCES IN THE DETERMINATION OF NI IN HUMAN ALBUMIN SOLUTIONS BY MEANS OF ID-ICP-MS

Size-exclusion chromatography (SEC) is used to separate biological molecules according to their size and to substitute unwanted ions and low molecular weight compounds in complex solutions with suitable buffers.

Since many of essential trace metals are strongly bound to proteins, the metal-protein complex can be separated by SEC from ions known to cause polyatomic or isobaric interferences.

Lyon et al. (1988b) used SEC to remove chloride ions from blood serum and eliminate a major interference ($^{40}Ar^{37}Cl^{+}$), affecting the determination of ^{77}Se. In a later paper, Lyon and Fell (1990) applied the same procedure to the determination of the isotopic composition of Cu in blood serum by ICP-MS. Copper analysis in serum suffered from the overlapping of the major Cu isotope, ^{63}Cu, with the polyatomic species $^{23}Na^{40}Ar$. This was completely eliminated after reduction of the sodium content by SEC.

The same approach could be used for the determination of other metals with known high affinity for specific proteins, such as Ni has for albumin (stability constant of the 1:1 Ni^{2+}-albumin complex: $10^{9.57}$ litres/mole,

Glennon and Sarkar, 1982). Most of the interferences in Ni analysis by ICP-MS are caused by the high Na and Ca content, which can be reduced by SEC. However, the proportion of serum Ni bound to proteins and that in low molecular fractions has not been definitely established and may vary between individuals. Therefore, ID would be required to provide quantitative results for total Ni content of the sample and allow for any incomplete recovery. This requires that at least two isotopes are made available for determination.

I investigated the use of this technique for the analysis of Ni in human albumin and plasma protein solutions (HAS, PPS), which are available in large amounts and contain higher concentrations of Ni than human serum or plasma, due to contamination during the production process (Diver et al., 1988; ch. 2).

3.2.1 Experimental

Instrumentation: Measurements of isotopic ratios and quantitative determinations of Ni were carried out on a PlasmaQuad inductively coupled plasma mass spectrometer (VG Elemental, Cheshire, UK). Instrumental settings and operating conditions are reported in Table 3.2.

Table 3.2 ICP-MS operating conditions.

Plasma		
R.f. power		
	Forward	1.3 kW
	Reflected	<10 W
Gas control		
	Auxiliary	0.6-0.7 l/min
	Coolant	13-14 l/min
	Nebulizer	0.75-0.85 l/min
Nebulizer	Meinhard type pumped at 0.7 ml/min	
	Spray chamber	Scott-type double bypass, water cooled
Ion sampling		
	Sampling cone	Nickel sampler (Nicone) with 1.0 mm orifice
	Skimmer cone	Nickel (001 Type) with 0.75 mm orifice
	Sampling distance	10 mm from load coil
Vacuum		
	Expansion stage	2.4 mbar
	Intermediate	$<1x10^{-4}$ mbar
	Analyser	3×10^{-6} - 4×10^{-6} mbar
Data aquisition:	Peak jumping	
	No. channels	1024
	No. of sweeps	20
	Points per peak	5
	Dwell time	80 µs

Reagents: ^{62}Ni, 98.83%, was purchased from AEA Technology, Oxford, UK. The mass analysis is shown in Table 3.3. Solutions of naturally abundant Ni were prepared from a stock standard solution of Ni 1000 mg/L in HNO_3 M (BDH, 'Spectrosol', BDH Ltd., Poole, UK). Disposable chromatographic columns, pre-packed with Sephadex G-25 M, (PD-10), were from Pharmacia LKB Biotechnology (Stockholm, Sweden). Nitric acid 65%, (Aristar, BDH) was further purified by subboiling in PTFE bottles. Ultrapure water was obtained by a four-stage purification using ion-exchange (Elgastat UHP, Elga, High Wycombe, UK).

Samples: Samples of HAS and PPS were obtained from the GRI Pharmacy or donated by manufacturers (Sec. 2.2).

Spiking procedure: Seven mg of ^{62}Ni were dissolved in 1 ml of concentrated HNO_3 and made up to 100 ml. Spiking solutions containing 0.7 mg/L and 7 mg/L of ^{62}Ni were obtained by appropriate dilution in 1% HNO_3. A volume of 15 ml of each protein solution was spiked with an appropriate amount of ^{62}Ni, chosen to obtain a 62/60 ratio in the sample close to 1. The spiking solutions were calibrated by reverse ID. Calibration solutions contained 100 µg/L of naturally abundant Ni and approximately 28 µg/L of ^{62}Ni.

Table 3.3 Mass analysis of enriched isotope preparations, as provided by manufacturer.

	Atom %				
Enriched isotope	58	60	61	62	64
^{62}Ni	0.32	0.63	0.13	98.83	0.09
^{61}NiO	1.93	3.41	93.61	1.06	-

Size-exclusion chromatography: To remove any Ni contamination, all PD-10 columns were washed with 20 ml of Na_2EDTA 69 µmol/L and rinsed with 30 ml of de-ionised water before use. A volume of 2.5 ml of each spiked albumin solution was loaded onto the column and eluted with 3.5 ml of de-ionised water. Samples were stored at +4°C until analysis and then further diluted with 1% HNO_3 to a final dilution of 1:10.

ICP-MS analysis:

Isotopic dilution analysis: Five measurements were carried out on each sample. The background signal obtained by analysing a blank solution (subboiled HNO_3 1%) was subtracted to all other measurements. The extent of mass discrimination affecting the measurement of the isotopic ratio 62/60 was evaluated at the beginning of every day by running a set of solutions of naturally abundant Ni and checked periodically during the day, running a standard solution every 5 samples.

The measured ratio (IR) between the spiked (A) and the reference (B) isotope in a sample corresponds to :

$$IR = (A_n + A_s) / (B_n + B_s)$$

where A_n and B_n are the masses of the spike and the reference isotopes in the sample before spiking and A_s and B_s are the masses of the spike

and the reference isotopes in the spike solution. From this equation the following formula was derived to calculate the concentration of Ni in the sample:

$$C = \frac{M_S (A_S - B_S\, IR)\, W}{A_S (IR\, B - A)\, V}$$

where C is the analyte concentration in the sample (µg/L or µg/g), M_S is the mass of the spike isotope (nmol), V is the volume (ml) or weight (mg) of the sample, A is the natural abundance of the spike isotope, B is the natural abundance of the reference isotope, A_S is the abundance of the spike isotope in the spike, B_S is the abundance of the reference isotope in the spike, W is the atomic weight of the element to be determined and IR is the measured ratio (spike isotope/reference isotope) corrected for mass discrimination where needed, after the spike addition.

The concentration of the spike solution was determined by reverse calibration and calculated according to:

$$C_S = \frac{(IR\, B - A)\, Q}{(1 - IR\, B_S / A_S)\, V_S}$$

where C_S is the concentration of the spike solution, Q is the amount of total Ni present (nmol), V_S is the volume of the spike (mL).

External calibration: Human albumin solutions were diluted 1:5 or 1:10

in HNO_3 1% and spiked with 50 µg/L In as internal standard. Three measurements were carried out on each sample and a blank solution (1% HNO_3) was subtracted to each measurement as described above. A set of standard Ni solutions prepared in HNO_3 1% was also analysed and the calibration curve and samples concentration were obtained using the instrument software.

3.2.2 Results and discussion

Preliminary analyses were carried out by quantitative ICP-MS using external calibration on HAS with a range of Ni concentrations, simply diluted 1:5 (Table 3.4). As expected, only the results obtained at mass 60 were in good agreement with results provided by GFAAS for all samples. The concentrations measured at mass 62 were highly degraded by the presence of polyatomic interferences, but in one sample, desalted on a PD-10 column before analysis, results were in close agreement with the concentration measured at mass 60 and by GFAAS.

These findings suggested that the desalting procedure could make the ^{62}Ni isotope available for ID. The results obtained for HAS with Ni content higher than 50 µg/L by means of three different techniques: ICP-MS with external calibration, ID-ICP-MS and GFAAS are compared in Table 3.5.

Table 3.4 Nickel concentrations determined at various masses in HAS diluted 1:5 by ICP-MS using external calibration. Sample LD1 was also desalted on a PD-10 column.

	ICP-MS				GFAAS
Mass:	58	60	61	62	
Sample:					
A, µg/L	27	21	12	4575	20.4
B, µg/L	12	8	12	10855	7.4
H, µg/L	107	95	139	11200	98
F, µg/L	74	66	85	7200	65
HMS, mg/L	0.39	0.36	0.43	8.5	0.39
E, mg/L	0.38	0.36	0.42	7.9	0.37
A1, mg/L	0.44	0.43	0.48	8.9	0.43
LD1, µg/L	70	65	80	67	64

Table 3.5 Comparison of different procedures for the determination of Ni in HAS: ICP-MS with external calibration; ID-ICP-MS and GFAAS (Ni concentrations > 50 µg/L, standard deviation in enclosure).

SAMPLE CODE	ICP-MS* ^{60}Ni		ID-ICP-MS#		GFAAS
KAG, µg/L	52	(2.0)	51	(1.6)	64
F, µg/L	66	(1.5)	64	(2.0)	65
H, µg/L	95	(1.5)	93	(1.4)	98
A1, mg/L	0.441	(0.003)	0.424	(0.002)	0.43
SHA1, mg/L	1.03	(0.02)	1.10	(0.003)	1.02

* dilution 1:10

SEC and dilution 1:10

The isotopic ratio was measured with a precision ranging from 3.3 to 0.3%, for Ni concentrations between 51 and 1100 µg/L. The results obtained with the three methods were in good agreement, confirming that SEC was effective in reducing the concentration of interfering species. However, sensitivity was insufficient to determine accurately Ni concentrations below 50 µg/L.

I attempted the direct determination of Ni concentrations lower than 50 µg/L in desalted samples of HAS and human serum, after protein precipitation with concentrated HNO_3. The Ni content in these samples was calculated for both ^{60}Ni and ^{62}Ni using external calibration and compared with results obtained by GFAAS (Table 3.6). The concentrations of Ni determined at mass 60 were still in agreement with the GFAAS results or the expected values, although generally lower, but definitely unreliable results were obtained from the ^{62}Ni isotope, indicating unresolved interferences and precluding ID.

The high results reported for ^{62}Ni in Table 3.6 could be due to poor resolution between a rather small ^{62}Ni signal and a quite large ^{63}Cu peak. The ^{63}Cu isotope accounts for 69% of the Cu concentration, which in HAS ranged from 64 to 239 µg/L, (ch. 2, Table 2.5) and in human serum from 500 to 1500 µg/L. Beauchemin et al. (1988a) also reported the overlapping between the ^{63}Cu and the ^{62}Ni peak in the analysis of two

Table 3.6 Determination of Ni at mass 60 and 62 by ICP-MS in: HAS, compared with values obtained by GFAAS; human serum samples, spiked with known amounts of Ni (Ni concentrations ≤ 50 µg/L, standard deviation in enclosure). Sodium content of the samples, determined by Flame Atomic Emission Spectrometry.

SAMPLE CODE	ICP-MS*		GFAAS	Na
	^{60}Ni	^{62}Ni		
	µg/L	µg/L	µg/L	mmol/L
I2	6.9 (0.2)	89 (50)	7.7	2.9
I4	8.0 (0.3)	321 (90)	8.5	3.0
I	9.3 (0.4)	485 (82)	13.5	2.4
L	11.0 (0.1)	632 (99)	13.6	2.8
A	21.0 (0.1)	539 (62)	20.4	2.2
HS**	4.1 (0.2)	1101 (103)		4.7
HS + 25 µg/L	25.1 (0.4)	870 (125)		4.2
HS + 50 µg/L	44.7 (1.2)	786 (121)		4.3

* SEC and deproteinization

** Human serum

marine reference materials with Cu/Ni ratios of 5 and 80, respectively, and aqueous solutions with comparable concentrations of Cu and Ni. This interference could only be removed by operating at an higher resolution level (Beauchemin et al., 1988a; 1988b).

Another possible cause of these abnormal results could be the presence of residual interferences from Na_2O and NaK species. The Na content of the samples presented to ICP-MS ranged from 2.2 to 3.0 mmol/L in HAS and from 4.2 to 4.7 mmol/L in serum samples (Table 3.6). Attempts to further reduce the Na content in the desalted fraction of serum samples by repeated runs on the PD-10 columns were not successful (Table 3.7). The concentration of Na in the eluate after 4 consecutive runs was only slightly reduced in comparison with that obtained after a single run (10.7 mmol/L). Therefore, I studied the elution profile of Na from the PD-10 columns and investigated some procedures for the complete elimination of Na ions from the samples.

In this experiment, I loaded a PD-10 column with 2.5 ml of human serum, followed by 12.5 ml of water. One ml fractions of the eluate were collected, starting as soon as the sample was loaded onto the column. Sodium content was determined in each fraction by flame atomic emission spectrometry (FAES) and the protein elution was monitored by determining the UV absorbance of each fraction at 285 nm. The elution

Table 3.7 Sodium content (standard deviation) in desalted serum fractions after repeated runs on PD-10 columns, corrected for dilution factor.

	Na, mmol/L	
Run	Column A	Column B
1	10.7 (0.90)	10.7 (0.05)
2	10.1 (0.05)	9.3 (0.10)
3	9.4 (0.50)	8.9 (0.40)
4	7.7 (0.10)	8.1 (0.25)

profile of Na and total protein are shown in Fig. 3.1. The two major peaks of Na and protein are well resolved, but small amounts of Na are eluted together with the proteins. The same experiment was then performed loading the column with 2.5 ml of a desalted serum fraction, obtained by running the same serum on a PD-10 column according to the procedure reported in Sec. 3.2.1. This second experiment indicated that the minor peak of Na was still eluted together with proteins and was identical to that observed in the first experiment (Fig. 3.1). I concluded that the residual fraction of Na could not be separated by SEC because of its association with proteins. Fogh-Andersen et al. (1993) studied the bound and net charge of human serum albumin at pH values between 4 and 9 and reported a -20 net charge per albumin molecule at pH=7, which could explain the electrostatic association of positive ions. Sodium ions associated with proteins could be partially substituted with alternative ions. In two separate experiments I added KCl or $RbNO_3$ (133 mmol/L) to a desalted serum sample and left it to equilibrate overnight at room temperature in the first case and for two hours on a rotating mixer in the second occasion. The samples were then run again through a PD-10 column and the eluate fractions were collected and analysed as described above. The elution profiles of Na were almost identical in both cases (Fig. 3.2). I observed a net reduction of the amount of Na eluted between

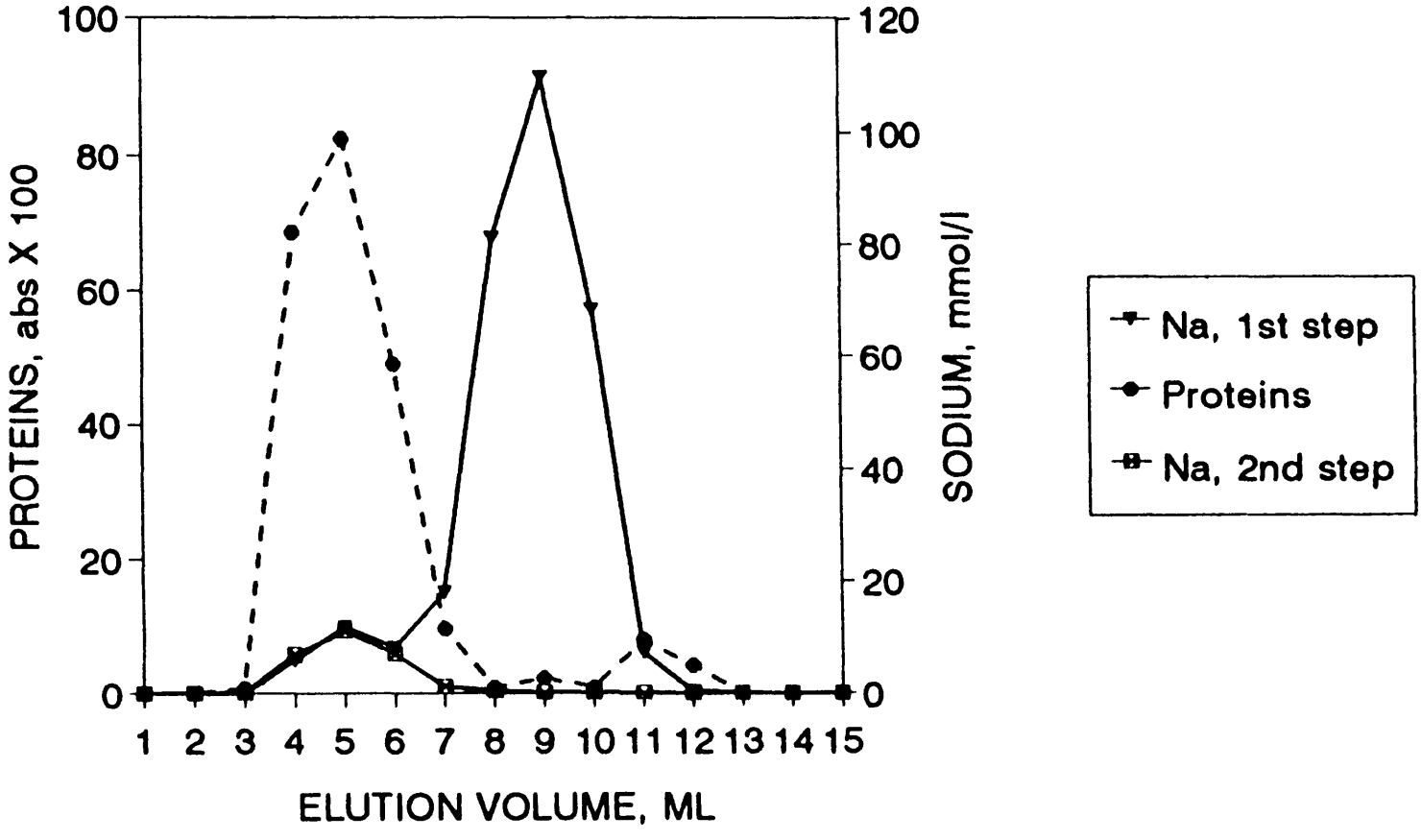

Fig. 3.1 Elution profile of Na and proteins from a serum sample in two consecutive desalting steps on PD-10 columns.

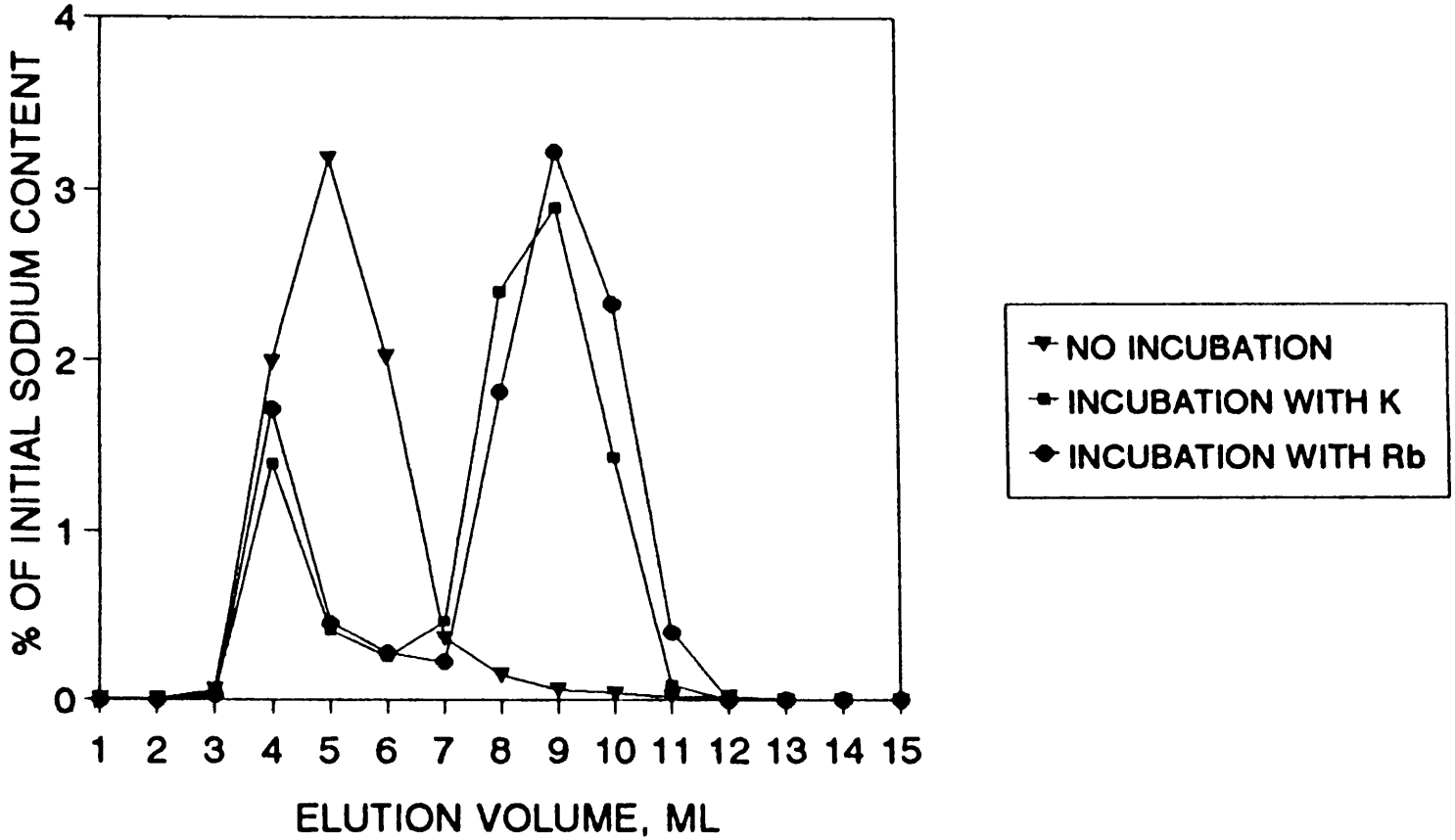

Fig. 3.2 Elution profile of sodium from PD-10 columns after incubation with alternative ions.

2 and 6 ml, as a consequence of the exchange of positive ions. I estimated the Na fraction still eluted together with the proteins as 2.1% and 2.5% of the initial Na content of the serum sample, for K and Rb incubation, respectively. In comparison, 8.4% of the initial Na content was eluted between 2 and 6 ml from non incubated serum samples. For a starting material with a Na concentration of 140 mmol/L, pre-incubation with competitive ions could reduce the Na concentration in the final solution to 1.4-1.7 mmol/L, taking into account a dilution factor of 2.058 for desalting and deproteinisation. According to results reported in Table 3.6, this concentration might still cause an interference at mass 62. In addition, high concentrations of salts and total dissolved solids caused problems when the samples were analysed by ICP-MS. A dilution of at least 1:10 of the samples was needed to avoid increasing imprecision and possible blockages due to salt deposits on the inner parts of the instrument.

In conclusion, although SEC proved valuable for the analysis of HAS with high Ni content, this method was limited by poor sensitivity and can suffer from residual interferences.

3.3 DETERMINATION OF NI ISOTOPES IN BIOLOGICAL MATERIALS BY ID-ICP-MS AFTER SOLVENT EXTRACTION

Solvent extraction has been widely used for the separation and preconcentration of metals from biological and environmental matrices. The procedure described in 1980 by the IUPAC Committee on Ni Toxicology (IUPAC, 1980), as part of the recommended method for the determination of Ni in biological fluids by GFAAS, has been accurately tested in an international trial and could be easily adapted to the treatment of solid samples as well. The complete removal of Na and Ca provided at least three Ni isotopes free from interferences and both *in vivo* enrichment and ID could be performed using the two minor isotopes.

3.3.1 Experimental

Samples: Analyses were carried out on human serum, plasma, red cells, urine and faeces specimens obtained from healthy volunteers and spiked with naturally abundant and enriched Ni. Red cells were separated from plasma by centrifugation at 2500 rpm for 15 min, washed three times with saline solution (0.9% NaCl, 'Aristar' grade, 99.99%) and lysed by addition of a volume of de-ionised water equal to the plasma volume.

Faeces were collected in a weighed plastic tub and homogenised after addition of a known amount of water. A 50 ml aliquot was weighed into a glass round bottom flask, freeze-dried and ground to a fine powder.

Nickel determination was also performed in the following control materials: Seronorm Trace Elements Urine batch no. 009024 (Nycomed Pharma, Oslo), recommended value 40 µg/L of Ni (range 36.4-42.8); BCR CRM 185, Bovine Liver and BCR CRM 186, Pig Kidney (Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium), indicative Ni content 1.4 µg/g and 0.42 µg/g, respectively.

Instrumentation: All measurements were carried out on a PlasmaQuad SX300 inductively coupled plasma mass spectrometer (VG Elemental, Winsford, Cheshire, UK) at the Rowett Research Institute, Aberdeen, Scotland. Samples were introduced by means of a peristaltic pump, using a Meinhard nebulizer. Instrumental settings and operating conditions were as reported in Table 3.2.

Reagents: Stable nickel isotopes, ^{61}Ni (93.61%, as NiO) and ^{62}Ni (98.83%, as metal) were obtained from AEA Technology, Oxford, UK. Mass analyses for both enriched materials were as reported in Table 3.3.

Ultrapure acids (HNO_3, 70%, $HClO_4$, 70% and H_2SO_4. 98%, 'Aristar'

grade;) methylisobutylketone (MIBK), 99.0%, ammonium tetramethylene-dithiocarbamate (ammonium pyrrolidine dithiocarbammate, APDC) 99.0%, and a Ni stock standard solution 1 g/L, all 'Spectrosol' grade, were from BDH Ltd. (Poole, UK). Nitric acid was further purified by subboiling in PTFE bottles. Ultrapure ammonia (NH_4OH, 25%) was from Normatom, ProLabo, France.

Potassium dihydrogen orthophosphate anhydrous (KH_2PO_4), 99.0%, disodium hydrogen orthophosphate (Na_2HPO_4), 99.5%, bromothymol blue (BTB) and chloroform ($CHCl_3$), all 'Analar' grade, were also from BDH.

Ultrapure water was obtained by a four-stage purification using ion-exchange (Elgastat UHP, Elga, High Wycombe, UK).

Working solutions: All working solutions were prepared according to the recommended IUPAC procedure (IUPAC, 1980). These included the acid mixture used for the digestion of samples, containing 3 parts of HNO_3, 1 part of $HClO_4$ and 1 part of H_2SO_4; solutions of BTB (0.4 g/L); APDC (2%), freshly prepared and purified by repeated extraction with MIBK; diluted NH_4OH (5%); diluted HNO_3 (1%) and a phosphate buffer containing 0.5 mol/L of KH_2PO_4 and 0.5 mol/L of Na_2HPO_4, pH=7, purified by extraction with APDC/$CHCl_3$.

Preparation and calibration of the ^{61}Ni and ^{62}Ni spike solutions. Stock solutions of ^{61}Ni (approximately 50 mg/L) and ^{62}Ni (approximately 70 mg/L) were prepared by dissolution of appropriate amount of materials in concentrated acid. Five mg of ^{61}Ni as ^{61}NiO were dissolved with 4 ml of HNO_3 and HCl 1+1 and the solution made up to 100 ml. The preparation of the ^{62}Ni solution was described in sec. 3.2.1. The actual concentration of the ^{61}Ni and ^{62}Ni spike solutions was determined by ICP-MS using a reverse calibration procedure (sec. 3.2.1).

Contamination control: To avoid inadvertent contamination of specimens, all glass- and plastic-ware was soaked overnight in nitric acid 20%, rinsed in ultrapure water and dried in a laminar flow hood. Manipulation of samples was also carried out in a laminar flow hood. Automatic pipette tips were rinsed three times with 20% nitric acid and ultrapure water before use.

Procedure: All samples were completely mineralised by acid-digestion. After addition of APDC, the Ni-complex was extracted in MIBK. The organic extracts were evaporated and the residues dissolved in concentrated HNO_3, then diluted to appropriate volume prior to analysis by ICP-MS. Stable isotopes for ID were added before digestion.

Acid-digestion: Five ml of water (blank), Ni standard solution, serum, plasma, red cells or urine and weighed amounts (0.2-0.5 g) of the solid samples (lyophilised faeces and tissues) were placed in 50 ml Pyrex conical flasks. Five ml of ultrapure water were added to solid samples.

For ID, appropriate amounts of the isotope solution were added to the samples at this stage and they were left to equilibrate for at least two hours at room temperature. The amount of isotope added was calculated to result in a ratio close to 1 for the chosen isotope pair.

Five ml of the acid mixture were added to each sample, the flasks covered and left overnight. The following day, the flasks were placed on a hot plate and digested according to the following temperature program: 110°C for 1 hr, 150°C for 2 hr, 200°C for 1 hr, 230°C for 30 min, 260°C for 30 min, 300°C until digestion was completed (about 2.5 hr). At this stage, the samples were clear, residual volume was about 1.5 mL and little or no more fumes were present. The samples were then left to cool down overnight in the laminar flow hood.

APDC/MIBK extraction: The pH range for the extraction of APDC-metal chelates has been widely investigated. Ni complexes were quantitatively extracted from aqueous solutions with pH values between 1 and 10 (Watson, 1971).

However, the IUPAC Committee observed poor stability of Ni complexes in the organic phase at low pH value and recommended the extraction to be carried out at pH=7 (IUPAC 1980). To adjust the pH of the digests to 7, I added to each sample: seven drops of BTB, 7.5 mL of ultrapure water, 3 mL of 25% NH_4OH and five drops of phosphate buffer. Addition of dilute (5%) NH_4OH continued until the colour of the solution changed from yellow to a green-blue shade comparable to that of an aqueous solution at pH=7 containing the same amount of BTB and phosphate buffer.

After addition of 1.25 mL of 2% APDC, each flask was mixed for 1 min and all samples were left standing for 15 min. Two mL of MIBK were added to each sample. Each flask was mixed by swirling for 2 min, then the content was poured in a 15 mL Pyrex glass tube. The organic phase separated without centrifugation and was transferred to a clean Pyrex glass tube. When external calibration was used, an exact volume of the organic phase (1.5-1.8 mL) was transferred.

Evaporation and dissolution: The organic extracts were evaporated to dryness using a heating block which was set at 120° C for 30 min and at 150°C for 45 min. The glass tubes were wrapped in Al foil to facilitate heat diffusion and shielded to reduce heat loss and avoid contamination.

Once dried, the extracts were left to cool at room temperature, then completely dissolved in 0.25 mL of HNO_3 followed by 0.75 mL of ultrapure water and well mixed to ensure complete dissolution. The samples were finally diluted with 8 mL of ultrapure water, mixed by inversion several times and stored at room temperature until analysis.

Analysis: A blank solution (2.8% HNO_3, corresponding to the acid concentration in the diluted extracts) was subtracted from all sample measurements to eliminate contributions from the background. The analyte concentration was calculated as described in section 3.2.1.

For quantitative analysis using external calibration, a set of Ni standard solutions prepared in HNO_3 2.8% was analysed together with the digests and a calibration curve and samples concentrations were obtained using the instrument software.

3.3.2 Results and discussion

Analytical performance: The mass spectra of a standard Ni solution, compared with extracted blank, urine and faeces samples, are shown in Fig. 3.3, 3.4, 3.5 and 3.6.

To assess the absence of interferences at the selected masses, the

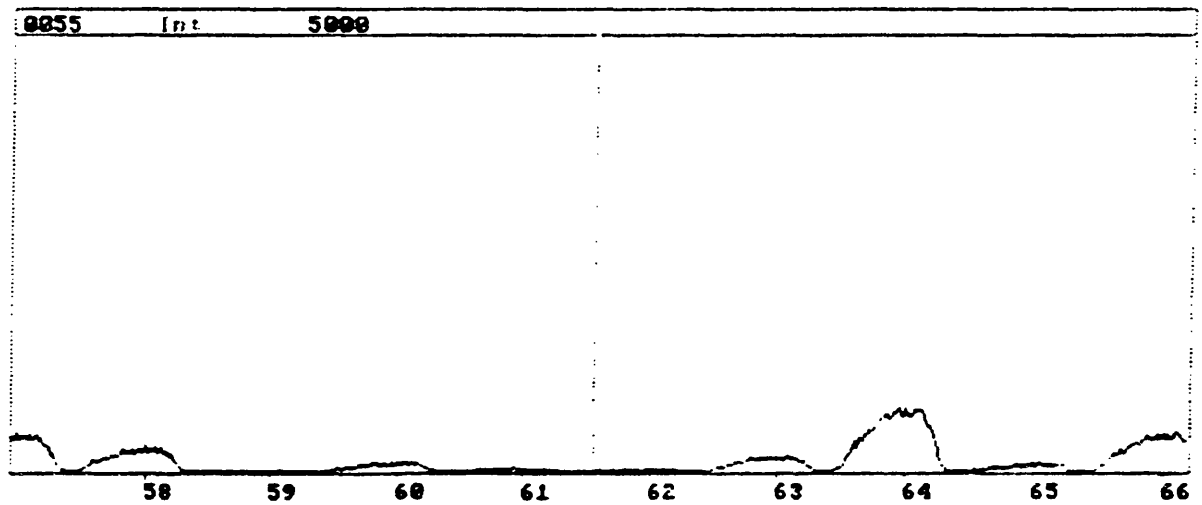

Fig. 3.3 Mass spectrum of blank (digested and extracted).

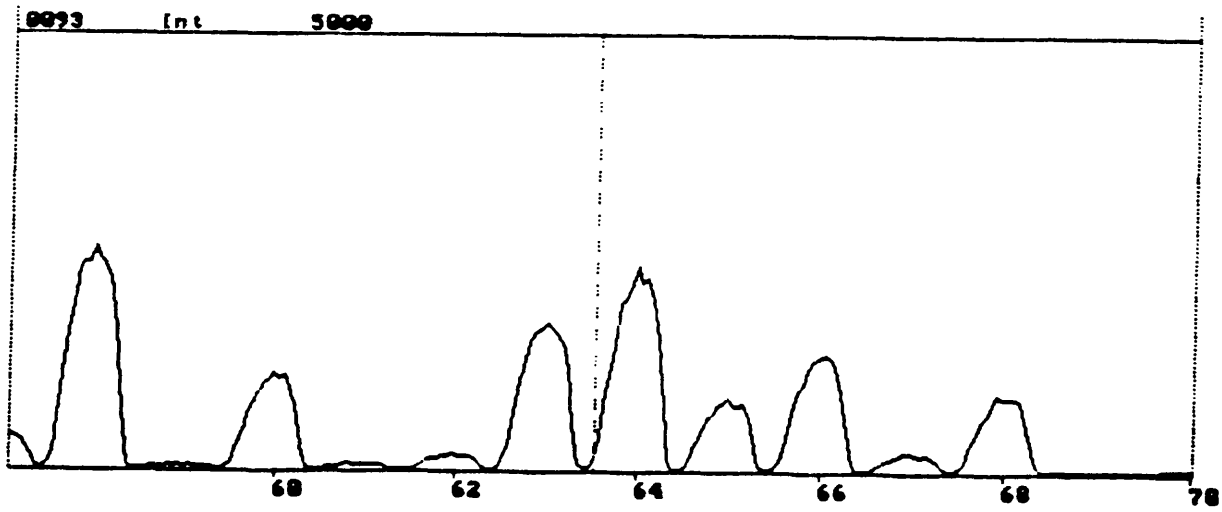

Fig. 3.4 Mass spectrum of human urine spiked with 50 µg/l Ni (digested and extracted).

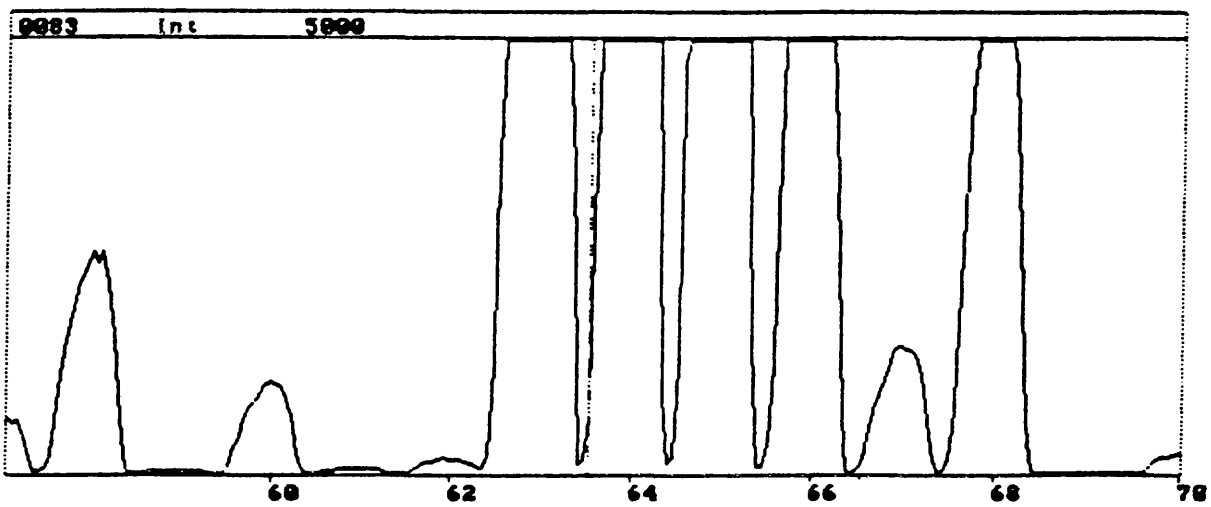

Fig. 3.5 Mass spectrum of human serum spiked with 50 µg/l Ni (digested and extracted).

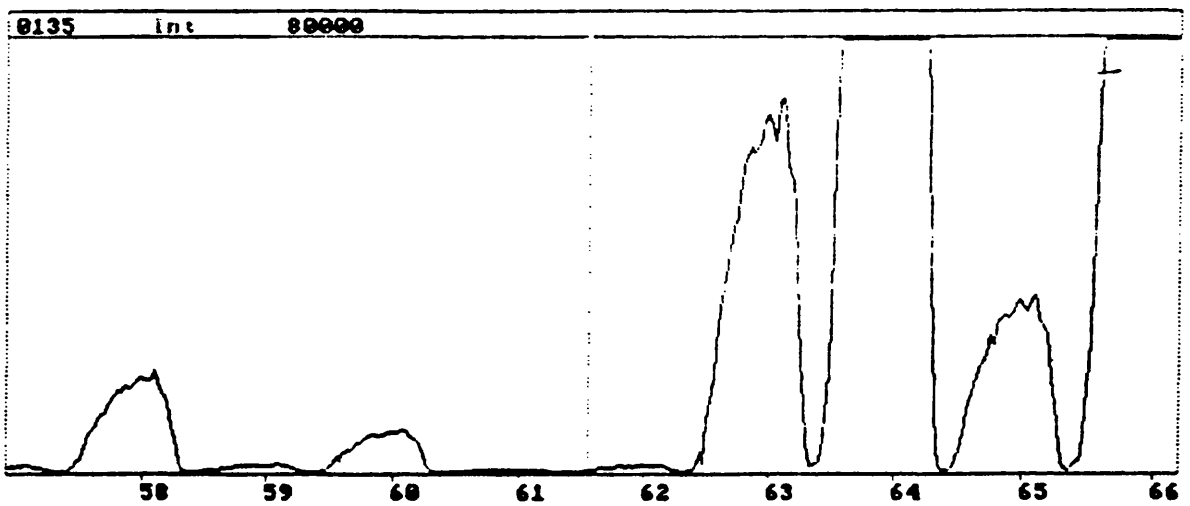

Fig. 3.6 Mass spectrum of human faeces (digested and extracted).

natural isotopic ratios between couples of isotopes were determined in various unspiked biological matrices (Table 3.8). The results obtained for urine, serum and faeces agreed well with the expected values for all the considered isotope pairs, within the limits of the experimental procedure, although a residual interference at mass 58 for serum samples was suspected. Due to the low natural abundance of ^{61}Ni, 61/58 and 61/60 ratios were only determined in faecal samples, which had a high Ni content. The analysis of lyophilised Pig Kidney provided good results for the isotopic ratio 62/60 but definitely lower values for the 62/58 and 60/58 ratios. Since Fe, Cu and Zn complexes with APDC are also extracted by this procedure, ^{58}Ni may suffer from overlapping with ^{58}Fe in samples with high Fe content, such as tissues (Fe concentration in CRM Pig Kidney: 299 µg/g). The ^{64}Ni isotope, with a natural abundance of only 1.14%, is almost always overwhelmed by the much more abundant ^{64}Zn in biological samples and was not considered in this study. A residual interference of ^{63}Cu on ^{62}Ni may also occur in samples with unfavourable Cu/Ni ratio. Beauchemin et al (1988a) observed this effect in samples of marine biological reference materials, which Ni/Cu ratio was 1:5 and 1:80, respectively. In this study, I only occasionally observed an overlap between the tail of the ^{63}Cu signal and the ^{62}Ni peak in serum samples with very low Ni concentration and standard solutions with similar Cu/Ni

Table 3.8 Isotopic ratios between different couples of Ni isotopes measured in unspiked biological samples after digestion and APDC/MIBK extraction. (All measurements corrected for mass bias; standard deviation in enclosure).

		Isotopic ratio				
		62/58	62/60	61/58	61/60	60/58
Expected ratio		**0.0540**	**0.1399**	**0.0184**	**0.0478**	**0.3861**
Sample	N					
Seronorm Urine	1	0.0534	0.01389	-	-	0.3844
		(0.0006)	(0.0035)	-	-	(0.0062)
Serum	1	0.0519	0.1394	-	-	0.3729
		(0.0016)	(0.0076)	-	-	(0.0104)
Pig kidney	1	0.0376	0.1405	-	-	0.2677
		(0.0004)	(0.0043)	-	-	(0.0053)
Faeces	5[a]	0.0556	0.1427	0.0188	0.0483	0.3891
		(0.0012)	(0.0016)	(0.0005)	(0.0015)	(0.0061)

[a] mean value from 5 different samples

concentrations (500/2.5). The 62/60 ratio was not affected in the Pig Kidney, which had a Cu concentration of 31.9 µg/g.

The results obtained for Ni added to various biological samples and reference materials, by means of ID with ^{62}Ni and ICP-MS (Table 3.9) indicated a satisfactory recovery from both serum (98 and 99.2%) and urine samples (98.5% and 103%) when the ratio 62/60 was used for calculation. Serum Ni values derived from the 62/58 ratio were slightly higher than expected (113% and 106%) which may be due to a residual Fe interference, whereas urine results agreed well with those derived from the 62/60 ratio (98% and 101%).

The analysis of Seronorm urine yielded values within the range of expected concentrations, although slightly higher than the target value (Table 3.9). However, as the manufacturer states that the control urine is prepared from normal urine spiked with 40 µg/L of Ni, a value slightly higher than 40 µg/L may be more accurate.

The determination of Ni in the reference material Pig Kidney gave very different values for the two isotopic ratios considered (Table 3.9). The result obtained from the isotopic ratio 62/58 was much higher than the indicated value. This was expected, since the analysis of unspiked materials (Table 3.8) suggested a possible overlap with ^{58}Fe. The result obtained from the 62/60 ratio is closer to the indicated value, although the

Table 3.9 Determinations of Ni in water, serum and urine spiked with natural Ni, using isotopic dilution with ^{62}Ni (µg/L ± RSD%).

MATRIX	ADDED Ni	Ni ISOTOPE RATIOS 62/58	62/60
WATER	25	25.6±2.5	25.2±2.7
	25	25.0±3.9	24.6±2.2
WATER	50	50.2±0.7	49.9±1.9
	50	48.8±2.7	48.5±2.2
SERUM (0.3 µg/L)	25	28.2±1.8	24.5±2.0
	50	52.8±1.9	49.6±3.1
URINE (2.0 µg/L)	25	27.4±2.5	27.9±2.1
	25	26.5±1.4	26.6±3.7
SERONORM URINE	40[a]	42.4±3.2	41.4±2.1
	40[a]	41.9±2.7	42.8±4.1
RM PIG KIDNEY	0.42[b]	1.08±1.3	0.61±1.4
RM BOVINE LIVER	1.4[b]	2.53±1.3	2.01±0.9

[a] Recommended value, range 36.4-42.8 µg/L.

[b] Indicative value, µg/g

difference still amounts to 0.19 µg/g (37%). The origin of this discrepancy is not clear, since the 62/60 ratio determined in unspiked Pig Kidney was in good agreement with the theoretical values, thus suggesting freedom from interferences. Since this material has not been certified for its Ni content yet, further analyses may be needed to gain some definite information. The analysis of the reference material Bovine Liver gave higher than expected isotopic ratios for both isotope pairs, which were attributed to high Fe and Cu content (214 and 189 µg/g, respectively).

The results of determinations performed on the same materials using external calibration are reported in Table 3.10. Ni was determined at mass 58, 60 and 62. The isotope ^{61}Ni, natural abundance 1.25%, did not provide a large enough signal. The determinations obtained at the various masses are in good agreement, but results were much lower than expected. I concluded that the sample pretreatment needed to remove interfering ions may lead to losses of material, during the digestion and/or extraction steps, and reckoned ID as mandatory for accurate results. The analysis of the lyophilised Pig Kidney gave higher results than expected.

Within run precision of ID-ICP-MS analysis of Seronorm urine was 1.4% and 1.7% (n=4), for the isotopic ratios 62/58 and 62/60, respectively. For serum samples, the coefficient of variation was 5.4% for the isotopic ratios 62/58 and 2% for the ratio 62/60, respectively (n=4, Table 3.11).

Table 3.10 Results of the determinations of Ni in water, serum and urine, spiked with natural Ni, using external calibration (µg/L ± RSD%).

MATRIX	ADDED	Ni ISOTOPES		
	Ni	58	60	62
WATER	50	41.0±3.3	41.7±2.1	38.0±3.2
		46.3±1.1	46.0±2.9	44.1±1.5
		38.0±0.5	38.0±1.6	30.4±9.9
SERUM (0.3 µg/L)	50	50.0±2.8	49.5±3.1	46.0±5.6
		55.7±1.1	52.3±0.8	50.5±3.1
		46.8±0.4	45.8±3.1	43.6±4.6
URINE (2.0 µg/L)	50	51.6±2.6	51.1±3.6	46.3±4.4
		48.3±1.4	47.1±2.3	44.9±8.8
SERONORM URINE	40[a]	30.8±1.3	31.2±3.2	29.0±1.4
RM PIG KIDNEY	0.42[b]	1.18±2.0	0.82±3.9	0.80±1.6

[a] Recommended Value, Range 36.4-42.8 µg/L.

[b] Indicative Value, µg/g

Table 3.11 Replicate determinations of Ni by means of ID-ICP-MS with ^{62}Ni in control urine and human serum. Nickel concentration derived from both 62/58 and 62/60 isotopic ratio.

MATRIX	TARGET VALUE (µg/L)	Ni CONCENTRATION (µg/L ± SD) 62/58	62/60
SERONORM URINE	40.0 (36.4-42.8)[a]		
1st Replicate		42.9±0.9	42.7±1.0
2nd Replicate		41.4±0.2	40.7±0.2
3rd Replicate		41.7±0.3	41.6±1.4
4th Replicate		41.6±0.5	41.4±1.1
OVERALL MEAN ± SD		**41.9±0.6**	**41.6±0.7**
SERUM	20.0		
1st Replicate		21.8±0.8	19.6±0.5
2nd Replicate		20.6±0.7	19.1±0.5
3rd Replicate		23.3±0.2	20.2±0.6
4th Replicate		23.4±0.6	19.7±0.5
OVERALL MEAN ± SD		**22.3±1.2**	**19.6±0.4**

[a] Recommended value (range).

Since the aim of this work was to establish a method that could be used for the determination of minor Ni isotopes acting as *in vivo* tracers of Ni metabolism, I determined ^{62}Ni added to biological materials of interest for metabolic studies, i.e. faeces, urine, blood serum or plasma and red cells (Table 3.12), using ID with ^{61}Ni. The recovery was between 97 and 106%.

Between-run precision was determined on serum and urine samples, spiked with ^{62}Ni, that were analysed in different days within three months (Table 3.13). Precision was 0.8% for serum samples and 2.4% for urine samples and the recovery of the added amounts of ^{62}Ni was 101% for serum and 98% for urine. In addition, 18 faecal samples were analysed in replicate in the same day to assess the within-day reproducibility of the determination of ^{60}Ni (n=5) and ^{62}Ni (n=13), (Table 3.14). The pooled standard deviation obtained for the ^{60}Ni determination was 0.12 µg/g (1.3%), for concentrations ranging from 4.6 and 15.1 µg/g. For ^{62}Ni, I obtained a pooled standard deviation of 0.19 µg/g (3.7%), for concentrations ranging from 0.92 and 10.2 µg/g.

Owing to the need for complete mineralisation of samples, the method is limited by high blank values that prevent the determination of low concentrations of naturally abundant Ni. In addition, the need to correct for blank values introduces an element of inaccuracy, due to variability between flasks and individual conditions of mineralisation. Estimated

Table 3.12 Determination of ^{62}Ni added to biological matrices by means of solvent extraction and ID-ICP-MS with ^{61}Ni

MATRIX	^{62}Ni ADDED µg/L	^{62}Ni FOUND µg/L	CV %	RECOVERY %
URINE	6.4	6.3	3.1	98
PLASMA	6.4	6.8	3.1	106
RED CELLS	6.4	6.6	3.0	103
FAECES[a]	0.078	0.076	2.3	97

[a]µg/g

Table 3.13 Between-day precision observed for serum and urine samples spiked with ^{62}Ni.

	SERUM + 9.6 µg/L ^{62}Ni			URINE + 26.5 µg/L ^{62}Ni		
	RESULT µg/L	SD µg/L	RSD%	RESULT µg/L	SD µg/L	RSD%
1st DAY	9.7	0.15	1.5	26.2	0.18	0.7
2nd DAY	9.7	0.11	1.1	26.7	0.13	0.5
3rd DAY	9.6	0.36	3.8	25.8	0.10	0.4
4th DAY				25.2	0.20	0.8
MEAN	9.7	0.08	0.8	26.0	0.62	2.4

Table 3.14 Within run precision of the determination of ^{60}Ni and ^{62}Ni in faecal samples using ID-ICP-MS with ^{61}Ni (µg/g dry weight ± standard deviation).

SAMPLE CODE	1st REPLICATE	2nd REPLICATE	DIFFERENCE
60 Ni			
1	8.71 ± 0.06	8.77 ± 0.04	-0.06
2	12.31 ± 0.04	12.09 ± 0.05	0.22
3	15.13 ± 0.04	14.90 ± 0.07	0.22
4	6.43 ± 0.03	6.24 ± 0.01	0.19
5	4.63 ± 0.04	4.63 ± 0.02	0.00
62Ni			
1	9.31 ± 0.01	9.28 ± 0.06	0.03
2	7.75 ± 0.01	7.44 ± 0.02	0.31
3	2.55 ± 0.01	2.64 ± 0.01	-0.09
4	0.92 ± 0.01	0.93 ± 0.00	-0.01
5	8.77 ± 0.03	8.07 ± 0.21	0.70
6	6.62 ± 0.02	6.39 ± 0.02	0.23
7	2.13 ± 0.01	2.05 ± 0.01	0.08
8	3.08 ± 0.01	3.13 ± 0.02	-0.05
9	8.98 ± 0.02	9.40 ± 0.02	-0.42
10	9.96 ± 0.02	10.19 ± 0.03	-0.23
11	1.26 ± 0.00	1.35 ± 0.00	-0.09
12	3.31 ± 0.01	3.31 ± 0.04	-0.01
13	2.85 ± 0.01	3.14 ± 0.01	-0.29

blanks values were about 3 µg/L. However, this is not a limiting factor for the determination of the minor isotopes, which are obviously less affected by contamination.

Since urine samples contain relatively little organic material, the digestion step may be avoided, thus drastically reducing the blank values and the time required for analysis. I compared the results obtained for eight urine samples at different concentration of ^{62}Ni using the complete procedure or omitting the digestion step (Table 3.15). The results were in good agreement and the observed differences were comparable with the analytical imprecision of the method.

Table 3.15 Determination of ^{62}Ni (µg/L) in urine by means of ID-ICP-MS with ^{61}Ni, after acid digestion and APDC/MIBK extraction or direct APDC/MIBK extraction

SAMPLE CODE	ACID DIGESTION + EXTRACTION	DIRECT EXTRACTION	DIFFERENCE (% DIFFERENCE)
BU1	34.4±0.2	34.3±0.2	0.1 (0.4%)
BU2	231.7±0.8	227.5±1.1	4.2 (1.8%)
BU3	149.3±0.8	144.7±0.3	4.6 (3.2%)
BU4	20.6±0.2	20.1±0.3	0.5 (2.5%)
BU5	10.7±0.1	10.7±0.3	0.1 (0.9%)
BU6	8.6±0.1	8.6±0.1	0.1 (0.6%)
BU7	10.0±0.1	10.1±0.0	-0.1 (- 1.0%)
BU8	9.1±0.1	9.2±0.1	-0.1 (-1.1%)

3.4 CONCLUSIONS

The investigation of separation procedures as a preliminary step to remove potential interfering species in ICP-MS proved successful for the determination of Ni in HAS and biological materials of human origin. Size-exclusion chromatography could be used to a certain extent to determine accurately the Ni content in HAS contaminated with Ni. The determination of lower Ni concentrations and the analysis of solid samples of interest in metabolic studies was obtained by using mineralisation of the samples, followed by chelation and solvent extraction. The accuracy and precision of the extraction method proved satisfactory for application to the investigation of Ni metabolism using ^{62}Ni as a tracer and ^{61}Ni for ID. Further investigations are needed to clarify residual interferences in the analysis of tissues, such as the reference materials Pig Kidney and Bovine Liver.

To date, this is the first procedure allowing the determination of three or more Ni isotopes in a variety of biological materials. Since other metals are coextracted, this procedure could be extended to the simultaneous analysis of other elements.

CHAPTER 4

ASSESSMENT OF NI METABOLISM IN MAN USING A STABLE ISOTOPE (^{62}Ni) AS A TRACER

4.1 INTRODUCTION

Knowledge of Ni metabolism is mainly derived from animal studies, most of them carried out using radioactive isotopes. Most of these investigations used extreme exposures, to enhance toxic effects and/or simulate work-place exposure. Different animal species were exposed to various Ni compounds under different experimental conditions. Gastrointestinal absorption was found to be poor (10% in dogs and 3-6% in rats). Single or repeated injections of Ni salts (intravenous, intraperitoneal or subcutaneous) led to the highest accumulation in the kidneys, endocrine glands, lung and liver. Most of the Ni was rapidly excreted in urine within 24 h after dosage (65-87%), the rest undergoing a much slower elimination (IPCS, 1991).

Onkelinx and co-workers (1973) proposed a mathematical model of Ni metabolism in rats and rabbits based on the observation of Ni concentrations in serum and urine for seven days after a single intravenous injection of $^{63}NiCl_2$. The doses were 82 µg/Kg for rats and 240 µg/Kg in rabbits. In both species they observed a rapid elimination of ^{63}Ni from blood during the first two days post-injection, whereas ^{63}Ni clearance was much slower between the third and seventh day. Rabbits eliminated 78% of the Ni dose in urine during the first day. Ni excretion in rats during three days post-injection was 78% in urine and 15% in faeces. Nickel excretion in bile, measured in two rabbits 5 h after injection, averaged 9.2% of the dose. In both species, the experimental results for Ni absorption and elimination fitted a two compartment model. The rate constants of Ni elimination were calculated as 0.108 and 0.088 h^{-1} in rats and rabbits, respectively, thus suggesting similar metabolic pathways.

In preliminary experiments intended to compare the results of investigations of Ni metabolism using radioactive and stable isotopes, Templeton et al. (1994) observed that rats excreted in urine 60 $\pm$ 17% of the dose of ^{63}Ni, within 80 h of the intravenous injection. An additional 5.4 $\pm$ 1.5% was excreted in faeces. The concentrations observed in serum over a period of 72 h fitted a double exponential decay as described by Onkelinx et al. (1973).

Jasim and Tjälve (1986) estimated the intestinal absorption of Ni in mice treated with ^{63}Ni to be 1.6%. Nickel was rapidly eliminated in urine and 97% of Ni excretion occurred within the first 24 h. The highest tissue concentrations of Ni were found in the kidneys, the lungs and the liver 24 h after oral administration, and in the spinal cord after 72 h.

More recently, Nielsen et al. (1993) reported a study of Ni metabolism in mice using a gamma-emitting isotope (^{57}Ni), which allows quantitative measurement of whole body retention to be made for as long as 130 h after dosage. Mice were given ^{57}Ni either orally or by intraperitoneal injection. The doses ingested were between 2.85 and 285 µg/Kg and the amounts given by intraperitoneal injection 10-fold lower. Gastrointestinal absorption was found to be between 1.7 and 10%. Whole body retention decreased rapidly after oral dosage and reached values ranging from 0.02 to 0.14% of the dose within 45-75 h after oral administration, then stabilised. In animals given ^{57}Ni intraperitoneally, whole body retention decreased to values ranging from 1 to 2% of the dose within 20-50 h and showed no further reduction. The highest Ni concentrations within the body were observed in the kidneys, carcass, lungs, testicles, liver and the spleen, 8 h after dosage, and in the carcass, kidneys, liver and lungs, after 20 h.

The findings of these and other studies suggest the absorption,

distribution and excretion of Ni to be species dependent. Differences in the experimental procedures, such as housing, feeding and the techniques of collecting and separating the excreta, all contribute to the variability of the results. Information obtained from animal studies may be of limited use for the understanding of the metabolism of Ni in humans.

Investigations on human volunteers have been restricted to dosing with naturally abundant Ni, due to ethical constraints preventing the use of long-lived Ni radioisotopes in humans (Spruits and Bongaarts, 1977; Cronin et al., 1980; Solomons et al., 1982; Gawkrodger et al., 1986; Sunderman et al., 1989).

Spruits and Bongaarts (1977) first reported an investigation of the concentrations of Ni in plasma and urine of a healthy, non-allergic volunteer, after ingestion of 5 mg of Ni as sulphate. Plasma concentrations were measured at 3, 6, 24, 30 and 48 h. The highest concentration (47 µg/L) was found 3 h after dosage, followed by a rapid decrease. Nickel concentration in urine remained higher than basal levels 104 h after dosage.

Cronin et al. (1980) administered doses of 0.6, 1.25 and 2.5 mg of Ni as sulphate to three groups of five women, all suffering from hand eczema due to Ni hypersensitivity, to investigate whether oral Ni intake caused exacerbation of symptoms. Within the next 24 h, blood samples were

collected using Pt needles and 24 h urine samples were voided into acid-washed containers. Serum Ni concentrations had a peak 2.5 h after dosage in all groups and ranged from 9 to 16 µg/L, 20 to 58 µg/L and 23 to 89 µg/L for the three levels of challenge. The amount of Ni excreted within 24 h ranged from 48 to 89 µg, from 62 to 253 µg and from 95 to 206 µg, for the three groups. All patients reported worsening of symptoms and the severity of response increased at higher Ni doses.

Solomons et al. (1982) estimated the effects of food and specific food components on the bioavailability of Ni for gastrointestinal absorption in man. An oral Ni dose (5 mg) was given to volunteers, after an overnight fast, and the rise in plasma Ni concentrations was compared with those obtained when Ni was given with food, various beverages, phytic acid, ascorbic acid, disodium EDTA and sodium iron EDTA. They observed a peak of 80 µg/L Ni 3 h after dosage in fasting subjects, but no change in Ni concentrations compared to basal levels when Ni was administered with food. Cow milk, coffee, tea, orange juice, ascorbic acid and EDTA salts also depressed Ni absorption, but Coca-Cola and phytic acid did not.

Gawkrodger and co-workers (1986) carried out a randomised double-blind crossover study on 26 sensitive subjects using three levels of challenge (0.4, 2.5 and 5.6 mg of Ni as sulphate), to clarify the effect of oral Ni intake on Ni dermatitis. Nickel concentrations in serum were still

about two and a half times higher than basal values 72 h after Ni ingestion in patients who had received doses of 2.5 and 5.6 mg of Ni (2.7 ± 0.2 µg/L and 2.6 ± 0.7 µg/L, respectively, vs. 1.2 ± 0.3 µg/L). However, clinical effects following the ingestion of the Ni dose in comparison with placebo were only seen in the group receiving the higher Ni dose. Serum Ni concentrations and urinary excretion were also measured in three healthy subjects after challenge with 2.5 mg Ni. The rise of serum Ni concentrations 3 h after dosage ranged from 30 times to only 5 fold the basal concentrations. Urinary excretion, measured in two post-challenge 24 h urine collections, was also variable and did not correlate with serum concentrations. The authors concluded that rather large doses of Ni would be necessary to induce adverse effects in Ni-sensitive patients. However, the possibility of small amounts of Ni in diet having an effect could not be ruled out, because the extent of Ni absorption and excretion varied considerably among individuals.

Sunderman et al. (1989) studied Ni absorption and its kinetics in ten human volunteers, who ingested doses of 12, 18 and 50 µg/Kg of Ni in two separate occasions after an overnight fast (1st experiment) or with breakfast (2nd experiment). Baseline values for serum Ni concentration and urinary and faecal excretion were obtained from pre-treatment samples. Plasma Ni concentration were measured after 1, 3, 7, 10, 24, 48

and 72 h. Ni excretion was determined in urine and faeces, collected for 4 days after dosage. Strict precautions to avoid contamination were taken during the study. Nickel absorption from the gastrointestinal tract was estimated as 27 ± 17% in fasting subjects and 0.7 ± 0.4% when Ni was ingested with food. Nickel elimination in both faeces and urine after four days amounted to 102 ± 8%, in the 1st experiment, and 104 ± 21%, in the 2nd experiment. The results of this study fitted a two compartment mathematical model similar to that developed for rats and rabbits by Onkelinx et al. (1973). The rate constants for Ni absorption, transfer and elimination did not differ significantly when Ni was administered with water or food. The average elimination half time for absorbed Ni was 28 ± 9 h. Renal clearance of Ni from the body was estimated as 8.3 ± 2.0 mL/min/1.73 m^2, when Ni was ingested with water, and 5.8 ± 4.3 mL/min/1.73 m^2, when Ni was given with food.

According to Santucci et al. (1994) factors controlling Ni metabolism within the human body can be altered, thus providing therapeutic treatment of Ni dermatitis. These authors reported the administration of gradually increasing oral Ni doses to eight Ni sensitive subjects for periods between 91 and 178 days. The treatment was well tolerated by all subjects who also reported clinical improvement of eczematous lesions not involving hands. The improved conditions of the treated patients was

attributed to reduction of gastrointestinal absorption of Ni and activation of renal excretion. Mobilisation of Ni deposits within the body was also suggested.

The early studies provided fundamental and invaluable knowledge on Ni handling by human beings, and the recent findings of Santucci et al. (1994) pose interesting questions. The limitations of these investigations arise from the use of naturally abundant Ni and the difficulties of separating the information obtained from the Ni dosage and that caused by other Ni sources. The dosage used, although safe, was much higher than physiological intake, in order to clearly identify the changes in Ni concentrations in biological fluids produced by the treatment. Both contamination of the samples and variable oral intake of Ni in the diet may have contributed to the scatter of results. Specimen contamination by Ni was less well controlled in the past. In some of the early studies samples were withdrawn using stainless steel needles (Solomons et al., 1982).

Stable Ni isotopes provide a means to overcome these problems. Less abundant isotopes, easily identified by their mass difference, can be used as tracers. Most confounding factors are thus excluded and lower dosage can be used to investigate the system with minimal perturbation. Stable isotopes are safe, since there is no radiation risk associated with their use and they can be administered even to vulnerable population groups, such

as infants and pregnant women. Since stable isotopes do not decay with time, the fate of the tracer can be followed for as long as needed and samples can be stored to be analysed at a convenient time and place. In the past, the applications of stable isotopes to metabolic studies was prevented by the need of extensive sample pretreatment and expensive instrumentation. Inductively coupled plasma mass spectrometry now allows the determination of the isotopic composition of a sample using less troublesome procedures than other MS techniques. Stable isotopes and ICP-MS have already been used for the investigation of iron absorption in women, before and during pregnancy (Whittaker et al., 1989; Whittaker et al., 1991; Barrett et al., 1992); Zn absorption in newborns (Ziegler et al., 1989; Serfass et al., 1989); Zn kinetics in adults with alcoholic liver disease (Lowe et al., 1993); Cu metabolism in healthy subjects and patients affected by Wilson's disease (Lyon et al., 1992).

Recently an attempt to investigate Ni metabolism in man using stable isotopes and ICP-MS has been reported (Templeton et al., 1994). In this study, one subject ingested a dose of 20 μg/Kg of ^{61}Ni and serum and urine concentrations of the isotope were followed for 96 h.

I report here the results of a more complete investigation of the absorption, distribution and excretion of Ni in humans, carried out on four volunteers, given an oral dose of ^{62}Ni as tracer.

4.2 EXPERIMENTAL

Subjects: the study was approved by the Research Ethics Committee at the Royal Infirmary, Glasgow, and volunteers gave their informed consent to the experiment.

The volunteers, two women (B.S., S.M.) and two men (L.W., P.G.), aged between 21 and 30 years, were healthy people recruited from the hospital staff, who had not suffered from any serious illness during the past five years; had regular bowel habit and had no past history of hypersensitivity to Ni. All subjects were non-smokers. The only medication taken were the contraceptive pill and oral antihistamine (one subject, S.M.). Diet was not restricted but guidance was provided about standard requirements in terms of fibres and nutrients. One subject (S.M.) was a vegetarian.

Organisation of metabolic experiments: Two separate experiments were carried out, each including a woman and a man (B.S. and L.W.; S.M. and P.G.). Each experiment lasted 5 days (Monday to Friday). A 24 h urine sample and a faecal sample were obtained from each subject the week before the experiment.

In each occasion, on Monday morning, a basal blood sample (10 mL)

was obtained by venepuncture from the two subjects after an overnight fast. The blood sample was withdrawn using an intravenous (i.v.) plastic cannula (Venflon), which was then left *in situ*.

Weight and height of each subject were recorded.

The bladder was emptied before drinking a 10 μg/Kg dose of ^{62}Ni (as nitrate in HNO_3 0.14 M) diluted in approximately 90 mL of water and adjusted to pH = 4. Together with the isotope dose, each subject ingested two gelatine capsules, each containing 10 radio-opaque barium sulphate impregnated polyethylene pellets to act as faecal marker (Cummings et al., 1976). The subjects were kept fasting for 2.5 h after the isotope ingestion and then allowed to return to their usual eating habits.

Further blood samples (10 mL) were obtained 0.5 h, 1.5 h, 2.0 h, 2.5 h, 3.0 h, 3.5 h, 4.5 h, 6.0 h (6.5 h in the second experiment) after isotope administration *via* the i.v. cannula. During the following four days a single blood sample was taken in the morning, 24 h, 48 h, 72 h and 96 h after the isotope ingestion, using standard stainless steel needles and plastic syringes.

Urine was voided in separate acid-washed plastic bottles during the following time intervals: 0-3 h, 3-6 h, 6-12 h, 12-24 h, 24-48 h, 48-72 h, 72-96 h, and 96-120 h. The faecal output of each of the four days after the isotope ingestion was collected directly into plastic containers.

Sample collection and storage: Blood samples were collected into 10 mL acid-washed plastic tubes containing 1.5 mg/mL K_2EDTA, free from Ni as assessed by GFAAS, and gently mixed several times by inversion. Packed cell volume (PCV) was measured on each sample using a microhaematocrit centrifuge. The exact volume of each sample was recorded and samples were centrifuged at 2500 rpm for 10 min. Plasma was separated using acid-washed plastic pipettes, then transferred into an acid-washed plastic tube and stored at -20°C. Red cells were washed three times with saline solution (0.9% NaCl, 'Aristar' grade, 99.99%), then diluted to the original volume of the blood sample with de-ionised water and stored at -20°C.

Urine samples were weighed and urine volumes determined multiplying by an average urine density of 1.02 g/mL. After thoroughly mixing, an aliquot of 100 mL was withdrawn and stored at -20°C.

Faeces were weighed and stored at 4°C. The number of radio-opaque pellets excreted in each sample was counted by X-ray visualisation using a Phillips BV 21 S Image Intensifier. Pellets were counted twice with the container in two different planes. Each stool was homogenised after addition of a known amount of water, depending on the size and consistency of the sample, using a Silverson Model L2R homogeniser. An aliquot of 50 mL was freeze-dried for 24 h or until constant weight was

reached. The lyophilised samples were then ground in a mortar to a fine powder and stored in plastic containers at room temperature.

Nickel analysis: Plasma, urine, red cells and faecal samples were analysed by ICP-MS, after isotopic dilution with ^{61}Ni, acid-digestion and extraction with APDC/MIBK, as described in Chapter 3.

Since impurities contained in H_2SO_4 made the highest contribution to blank values, the mixture for acid digestion was made up with 3.5 parts of HNO_3, 1 part of $HClO_4$ and only 0.5 part of H_2SO_4.

Urine samples (10 mL) were directly extracted with APDC/MIBK without digestion. Before extraction, samples were thawed, acidified to a final concentration of 1% HNO_3 and centrifuged at 2500 rpm to remove any precipitate.

Two aliquots of 0.1-0.2 g of each freeze-dried faecal sample were weighed directly into 50 mL conical glass flasks, diluted with 2.5 mL water and digested with 7.5 mL of acid mixture. One portion was used to determine the concentration of ^{62}Ni, given as tracer, and the other to measure the amount of ^{60}Ni, from which the amount of naturally abundant Ni, excreted in faeces as a result of dietary intake, could be calculated.

Each set of samples, for a given subject and matrix (plasma, red cells, urine and faeces), was analysed within the same day to reduce variability.

Control samples were prepared from pooled serum and urine spiked with known amounts of ^{62}Ni and analysed together with the plasma and urine samples obtained from the subjects of the experiment to check the accuracy of the results.

Concentration of spiking solutions: Three solutions of ^{61}Ni were prepared at 0.1, 0.5 and 5 mg/L and their concentration confirmed by means of ICP-MS using inverse calibration (see Chapter 3).

Additional analysis: Serum and urine creatinine was determined by the Jaffe' method, using commercial kits. Creatinine clearance, normalised according to body surface area (Burtis and Ashwood, 1994), was calculated with the following formula:

$$\text{Creatinine clearance} = \frac{\text{Volume of plasma cleared (mL)}}{\text{time (min) x standard surface area}}$$

i.e.

$$\text{Creatinine clearance} = \frac{\text{U-Crea (mmol/L) x U-Flow (mL/min) x1.73}}{\text{P-Crea (mmol/L) x BSA (m}^2\text{)}}$$

where U-Crea indicates urinary creatinine; U-Flow, the volume of urine (mL) which passes through the kidney in one minute; P-Crea, plasma

creatinine and BSA, the Body Surface Area, as obtained from standard nomograms (Burtis and Ashwood, 1994), using height and weight records.

4.3 RESULTS AND DISCUSSION

Details on each subject taking part in the study, including sex, age, weight, height, body surface area, average packed cell volume (PCV%) and total amount of ^{62}Ni received, are given in Table 4.1.

The observed daily output in faeces of radio-opaque pellets, dietary Ni and ^{62}Ni are reported in Tables 4.2, 4.3, 4.4 and 4.5, separately for each volunteer.

In all cases, the number of pellets given (20) was completely recovered in faeces within five days after ingestion, although patterns of faecal excretion differed. One of the volunteer, consuming a vegetarian diet, excreted 18 of the 20 pellets within 24 h, whereas the others required a longer time.

Radio-opaque plastic pellets were suggested by Cummings et al. (1976) as an ideal marker to estimate the transit time of digesta through the human gut. These pellets behave as closely as possible to natural dietary residues, because they do not interact with the gastrointestinal

Table 4.1 General details on volunteers taking part in the ^{62}Ni study

Subject	B.S.	L.W.	S.M.	P.G.
SEX	F	M	F	M
AGE, yrs	30	28	21	30
WEIGHT, Kg	66.2	77.5	59.2	86.5
HEIGHT, cm	162	186	169	179
BODY SURFACE AREA, m^2	1.70	2.02	1.68	2.06
PCV%	40	45	38	48
TOTAL ^{62}Ni DOSE, µg	670	760	571	879

Table 4.2 Daily faecal excretion, during metabolic experiment, of: total Ni and ^{62}Ni ingested with diet; total ^{62}Ni and ^{62}Ni from isotope ingestion (total ^{62}Ni -^{62}Ni ingested with diet). Subject: B.S..

Day	Radio-opaque Pellets	Sample weight		Concentration of total Ni, from diet		Excretion of total Ni, from diet	Excretion of ^{62}Ni (from diet)	Total ^{62}Ni concentration	Total ^{62}Ni excretion	Excretion of ^{62}Ni from isotope ingestion
	N	g ww	g dw	µg/g ww	µg/g dw	µg	µg	µg/g dw	µg	µg
0		118	34.0	3.27	11.41	388	14	0.42	14	0
2	9	136	27.8	1.79	8.75	243	9	9.30	259	250
3	8	97	23.9	3.02	12.18	291	11	7.60	182	171
4	2	81	23.3	4.31	14.98	349	13	2.60	61	48
5	1	66	22.4	4.96	14.60	327	12	0.93	21	9
Mean		99.6	26.3	3.47	12.38	320	12			
SD		28.1	4.8	1.21	2.63	55	2			
Total	20						45*		523*	478*

* basal sample not included

Table 4.3 Daily faecal excretion, during metabolic experiment, of: total Ni and ^{62}Ni ingested with diet; total ^{62}Ni and ^{62}Ni from isotope ingestion (total ^{62}Ni -^{62}Ni ingested with diet). Subject: L.W..

Day	Radio-opaque Pellets	Sample weight		Concentration of total Ni, from diet		Excretion of total Ni, from diet	Excretion of ^{62}Ni (from diet)	Total ^{62}Ni concentration	Total ^{62}Ni excretion	Excretion of ^{62}Ni from isotope ingestion
	N	g ww	g dw	µg/g ww	µg/g dw	µg	µg	µg/g dw	µg	µg
0	-	148	43.1	1.78	6.10	263	10	0.24	10	0
2	11	160	42.6	0.92	3.45	147	5	8.45	360	355
3	5	58	15.8	1.42	5.19	82	3	6.50	103	100
4	4	97	29.9	1.94	6.29	188	7	2.10	63	56
5	0	139	37.7	2.25	8.30	313	11	0.39	14	3
Mean		120.4	33.8	1.7	5.87	199	7			
SD		42.2	11.4	0.5	1.83	92	3			
Total	20						26*		540*	514*

* basal sample not included

Table 4.4 Daily faecal excretion, during metabolic experiment, of: total Ni and ^{62}Ni ingested with diet; total ^{62}Ni and ^{62}Ni from isotope ingestion (total ^{62}Ni -^{62}Ni ingested with diet). Subject: S.M..

Day	Radio-opaque Pellets	Sample weight		Concentration of total Ni, from diet		Excretion of total Ni, from diet	Excretion of ^{62}Ni (from diet)	Total ^{62}Ni concentration	Total ^{62}Ni excretion	Excretion of ^{62}Ni from isotope ingestion
	N	g ww	g dw	µg/g ww	µg/g dw	µg	µg	µg/g dw	µg	µg
0	-	26	7.0	2.69	10.00	70	3	0.39	3	0
2	18	339	75.7	1.03	4.63	351	13	3.31	251	238
3	2	150	35.9	1.78	7.45	267	10	3.00	108	98
4	0	216	50.1	1.56	6.70	336	12	0.36	18	6
5	0	172	46.2	1.60	5.95	275	10	0.22	10	0
Mean		219.3	43.0	1.49	6.18	307	11			
SD		84.4	24.9	0.32	1.20	42	1.5			
Total	20						45*		387*	342*

* basal sample not included

Table 4.5 Daily faecal excretion, during metabolic experiment, of: total Ni and ^{62}Ni ingested with diet; total ^{62}Ni and ^{62}Ni from isotope ingestion (total ^{62}Ni -^{62}Ni ingested with diet). Subject: P.G..

Day	Radio-opaque Pellets	Sample weight		Concentration of total Ni, from diet		Excretion of total Ni, from diet	Excretion of ^{62}Ni (from diet)	Total ^{62}Ni concentration	Total ^{62}Ni excretion	Excretion of ^{62}Ni from isotope ingestion
	N	g ww	g dw	µg/g ww	µg/g dw	µg	µg	µg/g dw	µg	µg
0	-	107	34.6	2.92	9.01	312	11	0.33	11	0
2	5	167	44.7	1.34	5.02	224	8	3.11	139	131
3	3	30	11.8	2.46	6.28	74	3	9.19	108	105
4	7	78	23.6	2.14	7.07	167	6	10.08	238	232
5	5	499	128.3	1.71	6.67	855	31	1.31	167	136
Mean		176	48.6	2.11	6.81	326	12			
SD		187	46.2	0.60	1.45	308	11			
Total	20						48*		652*	604*

* basal sample not included

system and their specific gravity, ranging from 1.3 to 1.6, is close to that of faeces. There are no risks associated with their use and their detection is simple and safe. The average transit time (ATT) of digesta in subjects given a single dose of pellets is calculated as:

$$ATT = \Sigma (x_i t_i)/\Sigma x_i$$

(where x_i is the number of pellets excreted in each day and t_i the time (h) elapsed since marker ingestion). In this study I observed ATT values of 55.2, 39.6, 26.4 and 68.4 h, for B.S., L.W., S.M. and P.G., respectively. This corresponds to an average value of 47.4 h (SD 18.3; SEM: 9.1) and, if I exclude the vegetarian subject, 54.4 h (SD: 14.4; SEM: 8.3). In a group of 6 healthy young men eating a free diet, Cummings et al. (1976) observed an average ATT of 55.2 h (range 16.8-96), which decreased to 38.4 h when additional fibre was included in the diet (n=5).

In all volunteers, the pattern of excretion of the faecal marker closely followed that of ^{62}Ni (Figs. 4.1 - 4.4). Therefore, I am confident that the recovery of ^{62}Ni not absorbed from the intestine was also complete.

The average concentration of dietary Ni observed in faeces of all subjects, (except the basal sample of subject S.M., which was reckoned incomplete), was 2.2 $\pm$ 1.1 µg/g wet weight (w.w., n= 19; range: 0.9-5.0) and 7.9 $\pm$ 3.3 µg/g dry weight (d.w., n=19; range: 3.5-15). The average percentage of dry mass was 27.8 $\pm$ 4.4% (n=19, range 20.5-39.2).

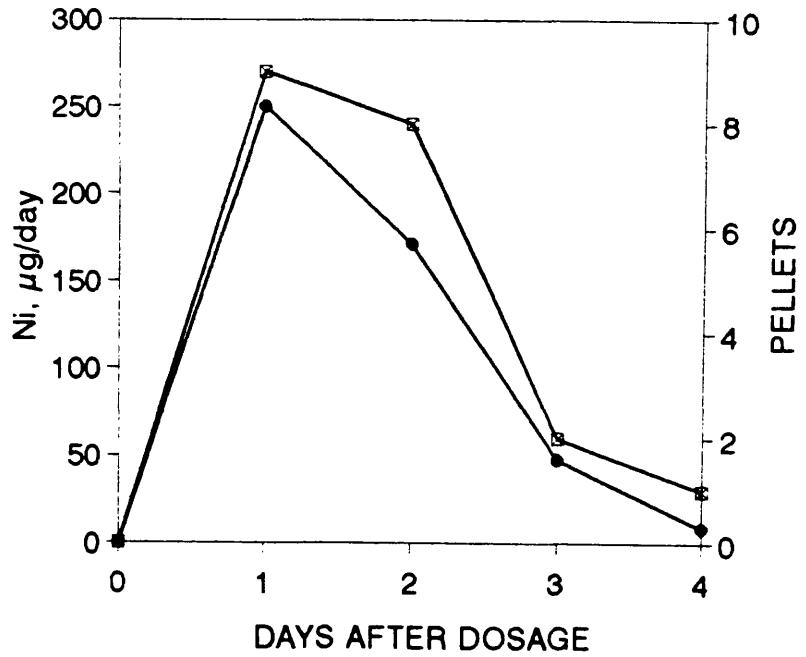

Fig. 4.1 Faecal excretion of ^{62}Ni (circles) and radio-opaque pellets (squares) after dosage (B.S.).

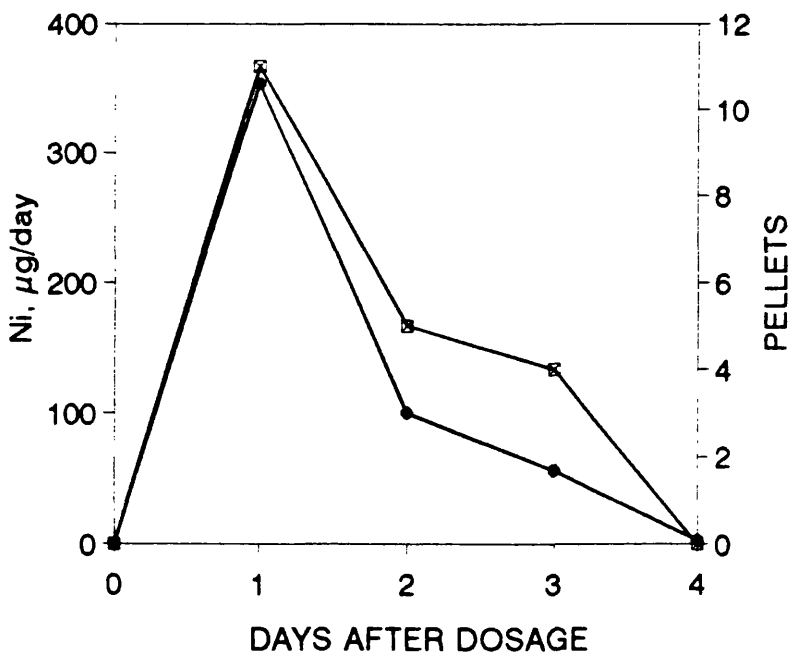

Fig. 4.2 Faecal excretion of ^{62}Ni (circles) and radio-opaque pellets (squares) after dosage (L.W.)

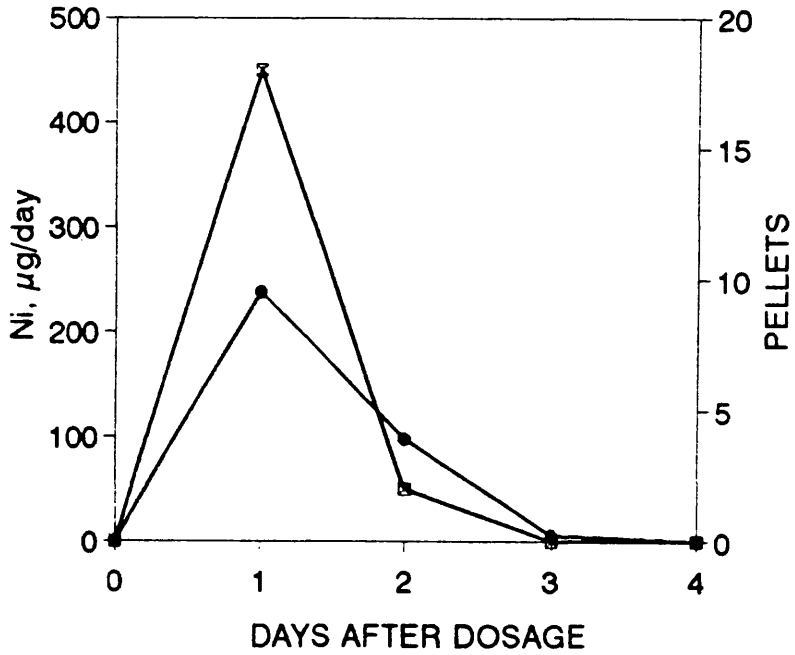

Fig. 4.3 Faecal excretion of ^{62}Ni (circles) and radio-opaque pellets (squares) after dosage (S.M.).

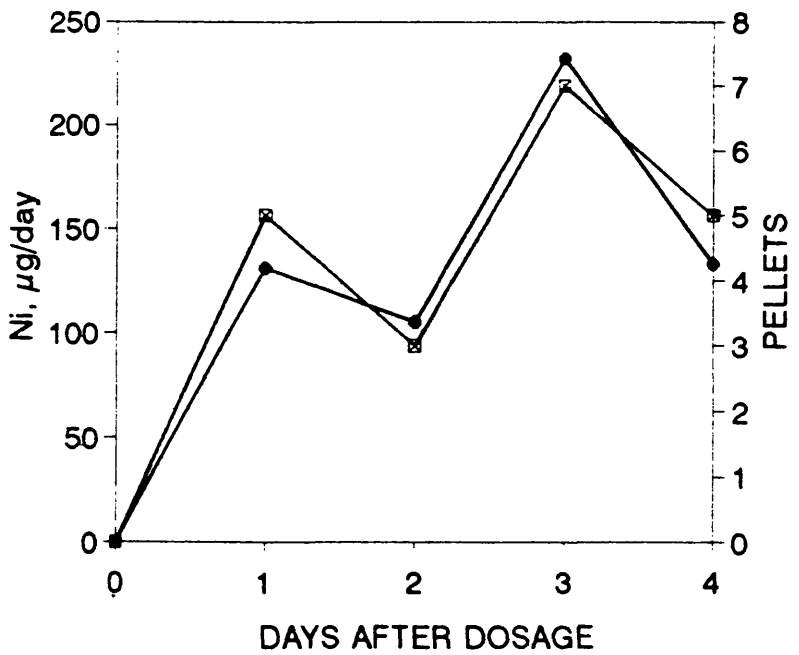

Fig. 4.4 Faecal excretion of ^{62}Ni (circles) and radio-opaque pellets (squares) after dosage (P.G.)

Daily faecal excretion of naturally occurring Ni averaged 255 ± 92 µg/day, (n=18; range: 74-388; 1 outlier excluded). Average individual Ni excretion, computed from the mean value of daily Ni excretion over five days, for the four subjects, was 288 ± 60 µg/day (n=4, range 199-326). Nodiya (1972) observed an average excretion of Ni of 258 ± 23 µg/day (range 219-278) in three-day faecal collections from ten Russian students consuming a school diet. Horak and Sunderman (1973) measured Ni in three-day collections of faeces from ten North American adults consuming a free diet. They reported an average Ni faecal concentration of 3.3 ± 0.8 µg/g w.w. (range 2.1-4.4); 14.2 ± 2.7 µg/g d.w. (range 10.8-18.7) and daily faecal excretion 258 ± 126 µg/day (range 80-540). The average concentration of Ni determined by Sunderman and co-workers (1989) in two-day faecal collections from ten subjects in the USA was 1.5 ± 0.5 µg/g w.w. (range: 1.0-2.2) and average daily faecal excretion was 158 ± 75 µg/day (range: 69-289).

The average daily excretion of Ni in faeces observed in this study is in agreement with previous reports and consistent with the estimate of dietary Ni intakes in the UK. Smart and Sherlock (1987) reported that people in the U.K. may ingest between 140 and 150 µg/day of Ni in food, with an additional 100 µg/day released from cooking utensils.

The concentration of naturally abundant Ni in faeces was quite high and rather variable on a day-to-day basis (Tables 4.2-4.5). This variability may have affected the estimate of the gastrointestinal absorption of Ni in experiments where volunteers ingested naturally abundant Ni. The use of a tracer should eliminate these problems. However, due to the high content of total Ni in human faeces, the amount of ^{62}Ni naturally present is not negligible and could still affect results, although to a minor extent. Therefore, I calculated the amount of ^{62}Ni from the diet as a percentage (3.66%) of the content of naturally abundant Ni, measured in the faecal samples using the isotope ^{60}Ni, and corrected the data of ^{62}Ni excretion accordingly (Tables 4.2-4.5). The fraction of ^{62}Ni from the diet ranged between 5.1% and 13.2% of the total amount of tracer excreted in faeces.

Faecal excretion of the tracer in the four volunteers averaged 66.9 $\pm$ 4.9% (range 59.9-71.3) of the dose and the absorbed fraction was calculated to be 33.1 $\pm$ 4.9%.

The plasma concentrations of ^{62}Ni observed in each of the four volunteers at definite time intervals (Table 4.6) are plotted against time in Fig. 4.5. Although large individual variability has been reported in earlier studies, the set of results observed in this group of subjects at each time were in close agreement (Table 4.6). Plasma ^{62}Ni had a peak between 1.5 and 2.5 h, at concentration ranging between 16.7 and 21.3 µg/L.

Table 4.6 Plasma ^{62}Ni concentrations (µg/L) at definite times after dosage in four volunteers. Mean and standard deviation of all of the concentrations observed for each time are also reported.

Time (hrs)	BS	LW	SM	PG	**Mean**	SD
0	0	0	0	0	**0**	
0.5	15.0	6.6	4.2	4.2	**7.5**	5.1
1.5	18.0	15.3	19.2	20.4	**18.2**	2.2
2.0	16.3	16.7	19.7	21.3	**18.5**	2.4
2.5	16.3	16.4	20.8	-	**17.8**	2.6
3.0	15.1	16.3	20.1	18.4	**17.5**	2.2
3.5	13.7	15.8	19.7	16.6	**16.5**	2.5
4.5	11.7	12.8	17.3	14.3	**14.0**	2.4
6.0	10.2	10.5			**10.4**	0.1
6.5			12.4	11.2	**11.8**	0.6
24	4.1	3.5	3.8	3.1	**3.6**	0.4
48	2.7	1.6	1.6	2.5	**2.1**	0.6
72	1.9	1.0	0.8	1.6	**1.3**	0.5
96	-	0.7	0.5	1.0	**0.7**	0.3

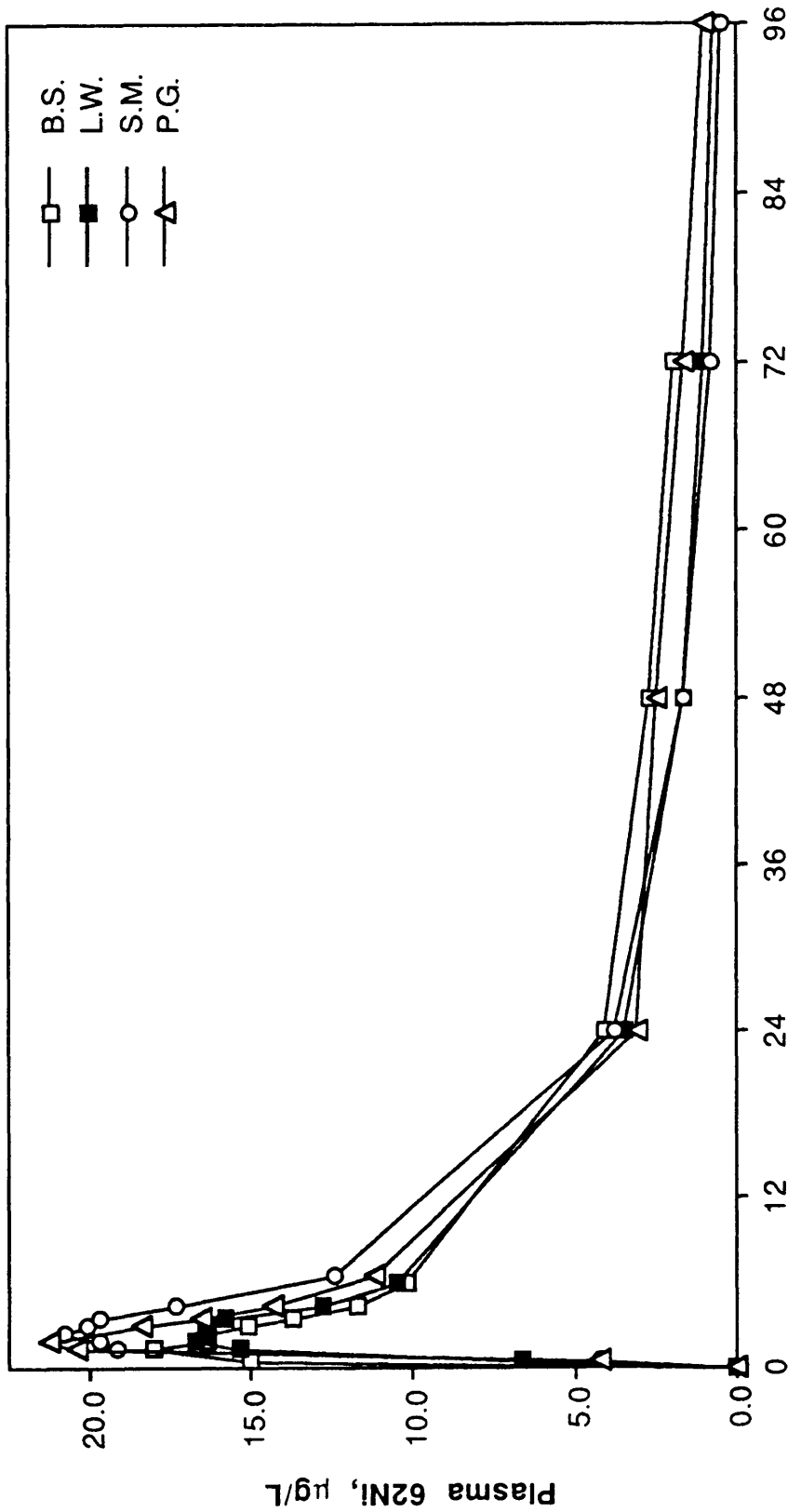

Fig. 4.5 Changes in plasma concentrations of 62Ni after dosage

Other studies reported the highest plasma concentration to occur at 2.5h or 3h (Spruits and Bongaarts, 1977; Cronin et al., 1980; Solomons et al., 1982; Gawkrodger et al., 1986; Sunderman et al., 1989). However, only Solomons et al. (1982) investigated serum Ni concentrations between 0 and 2.5 h. In a recent study, Templeton et al. (1994) also observed the peak of serum Ni concentrations at 2.0 h in a volunteer who ingested a dose of ^{61}Ni and had blood samples at 30 min intervals for 3 h after dosage. Clearance of ^{62}Ni was rapid and 24 h after ingestion plasma concentrations had dropped to between 3.1 and 4.1 µg/L. However, ^{62}Ni was still detectable in plasma 96 h after dosage, at concentrations ranging from 0.5 and 1 µg/L, comparable with values expected for plasma Ni in unexposed subjects.

The effect of contamination with naturally abundant Ni from the needles and other sources on the results of investigations of Ni absorption in blood is shown in Fig. 4.6. Total Ni, determined in the plasma samples by GFAAS, was generally higher than the concentration of ^{62}Ni, determined by ICP-MS. All results are transformed in µmol/L, as data expressed on a mass basis are biased by the difference in mass between the single isotope (mass 62) and total Ni, which is a mixture of five isotopes (average mass 58.71). To compare the results of determinations of ^{62}Ni and total Ni in plasma of rats, Templeton et al. (1994) adopted stringent

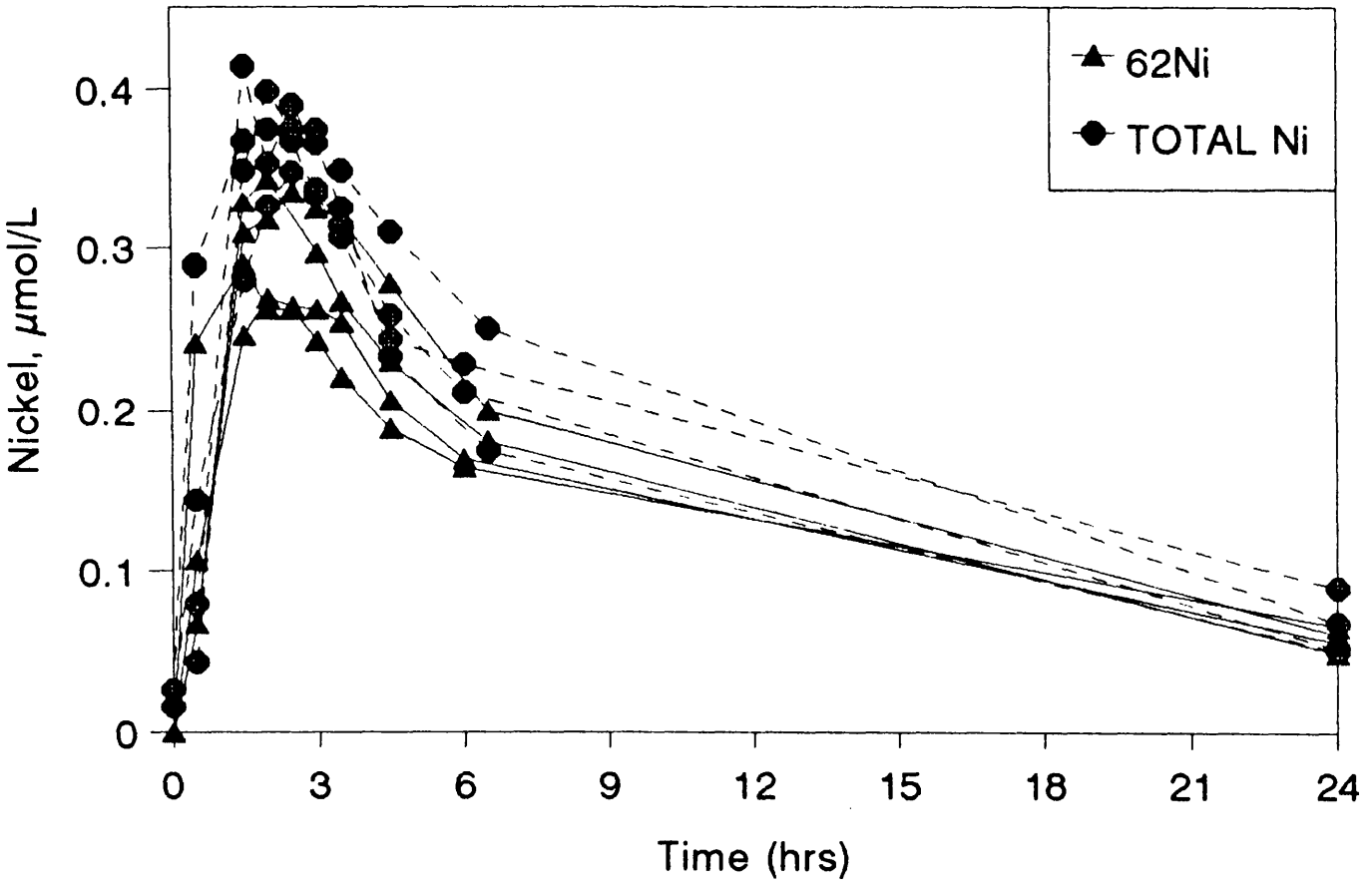

Fig. 4.6 Plasma concentration of 62Ni and total Ni after dosage, measured by ICP-MS and GFAAS, respectively (all subjects).

contamination control, including flushing the stainless steel needles used for blood sampling with 3 mL of blood before sampling.

Analysis of the results obtained for plasma ^{62}Ni concentrations, by means of a commercial pharmacokinetics programme (SIFAR, Milano, Italy), suggested that the log-concentration vs. time plot was best fitted by a function including three-exponential components. The values derived for the area under the curve (AUC), elimination half-time and ^{62}Ni clearance using this mathematical approach, are reported in Table 4.7 for the four volunteers. The overall inter-individual variation of the absorption and clearance of ^{62}Ni from the blood, evaluated from the dispersion of the AUC values, was only 10%. Average elimination half-time was 25.5 ± 4.4 h and Ni clearance from plasma was 10.4 ± 2.1 mL/min, as compared with 28 ± 9 h and 8.3 ± 2.0 mL/min observed by Sunderman et al. (1989).

The concentrations of Ni in serum and in whole blood have been reported to be similar in unexposed subjects (Hopfer et al., 1985). *In vitro* studies of cellular uptake of Ni suggest that high concentrations of albumin and aminoacids may prevent Ni transport across the cellular membrane of erythrocytes (Niebor et al., 1984, 1988). I have attempted to assess the distribution of Ni between red cells and plasma *in vivo*. The concentration of ^{62}Ni was determined in red cell samples taken from two

Table 4.7 Information derived from mathematical fitting of the plot log-plasma ^{62}Ni concentrations vs. time.

	Peak Conc. µg/L	Peak time h	AUC* h·µg/L	Elimination half-time h	Total ^{62}Ni clearance ml/min
B.S.	18.0	1.5	426	28.8	7.4
L.W.	16.7	2.0	340	24.0	12.3
S.M.	20.8	2.5	366	20.0	10.8
P.G.	21.3	2.0	408	29.2	11.1
MEAN	**19.2**	**2.0**	**385**	**25.5**	**10.4**
SD	**2.2**	**0.4**	**39**	**4.4**	**2.1**
CV%	**11.5**	**20.4**	**10**	**17**	**2.0**
MIN	16.7	1.5	340	20.0	7.4
MAX	21.3	2.5	426	29.2	12.3

*Area Under the Curve: log-^{62}Ni concentration vs time

of the volunteers (S.M. and P.G.) after ingestion of the isotope. In both cases, the concentrations of ^{62}Ni in red cells were much lower than that in the respective plasma samples. Unfortunately, the concentrations in the haemolysates presented to the ICP-MS were too low for acceptable analytical reliability. Although we require more precise analyses, the pattern of ^{62}Ni concentrations in red cells obtained for one subject are presented in Table 4.8. The uptake of ^{62}Ni by erythrocytes occurs slowly and reaches a peak of around 3.0 µg/L at about 48 and 72 h after ingestion.

Creatinine clearance and intra-individual variability for all subjects were within the expected reference range for healthy subjects (94.2 ± 12.7 mL/min/1.73 m^2; Gowan and Fraser, 1988), thus reflecting normal renal function and completeness of urinary collections. A lower day-to-day variability was observed for the vegetarian subject (S.M.), due to the lack of dietary creatinine intake. Individual average daily urinary volume ranged from 1015 to 1755 mL and average urinary flow was between 0.71 and 1.22 mL/min. The values observed for urinary volume, flow, creatinine excretion, serum creatinine and creatinine clearance for the four subjects are reported in Appendix.

Urinary concentrations and daily excretion of ^{62}Ni for all subjects are reported in Table 4.9. Daily and cumulative urinary excretion from all

Table 4.8 Concentrations of ^{62}Ni measured in erythrocytes of one subject (S.M.) at definite time intervals after dosage.

Time (h)	^{62}Ni concentration (µg/L erythrocytes)
0	0
0.5	0.3
1.5	0.3
2	0.4
2.5	0.7
3	0.6
3.5	0.6
4.5	0.8
6.5	0.9
24	2.5
48	2.9
72	2.7
96	2.1

Table 4.9 Concentration of ^{62}Ni in urine and absolute urinary excretion of ^{62}Ni for the four subjects during the five days of the experiment. Excretion and cumulative excretion of ^{62}Ni as a percentage of the absorbed dose.

	Urinary concentration, µg/l				Urinary excretion, µg				Excretion, %				Cumulative excretion, %			
Subjects	BS	LW	SM	PG	BS	LW	SM	PG	BS	LW	SM	PG	BS	LW	SM	PG
Time intervals																
0-3	34	192			3.1	27			1.6	11			1.6	11		
3-6	230	63	106	316	20	23	61.5	52	10.4	9.3	26.9	18.7	12.0	20.0	26.9	18.7
6-12	147	36	57	113	5.6	22.5	21.5	21	2.9	9.1	9.4	7.4	14.9	29.1	36.3	26.1
12-24	20	80	64	71	25	22.5	31	21	13	9.1	13.5	7.6	27.9	38.5	49.8	33.7
0-24	36.8	67.4	79.0	145	53.7	95	114	94	27.9	38.5	49.8	33.7	27.9	38.5	49.8	33.7
24-48	11	30	37	35	15.5	43	42	32	8.1	17.5	18.3	11.5	36.0	56.0	68.1	45.2
48-72	8.6	12	14	20	9.9	15	18	18	5.2	6.1	7.9	6.7	41.2	62.1	76.0	51.9
72-96	10	3.6	3.7	10	9.0	7.0	8.2	13	4.7	2.7	3.6	4.7	45.8	64.8	79.6	56.6
96-120	9.2	4.0	2.1	6.9	8.6	9.0	5.6	8.9	4.5	3.7	2.4	3.2	50.4	68.5	82.0	59.8
TOTAL					97	169	188	166	50.4	68.5	82.0	59.8				

subjects are plotted against time in Figs. 4.7 and 4.8 as percentage of the absorbed dose. The results show a large inter-individual variability in Ni excretion, only partially explained by the differences in urinary volumes and flows. Five days after ingestion, the elimination of Ni was not complete in any of the subjects. The concentrations of ^{62}Ni in urine collected on the fifth day (2.1 to 9.2 µg/L) was still higher than the concentrations found in urine of unexposed subjects. The percentage of the absorbed dose excreted in urine averaged 65.2 $\pm$ 13.4% (range 50.4-82.0).

The results of this metabolic study are summarised in Table 4.10, which gives the absorbed, excreted and retained fractions of the dose of ^{62}Ni found for each subject. The ^{62}Ni dose was relatively small and comparable with the amount of Ni that can be ingested with diets rich in certain types of food such as dark chocolate, nuts and soy products. This is the lowest amount used up to now in investigations of Ni metabolism in humans. Doses of 0.4 and 0.6 mg Ni have been administered during studies of clinical responses of Ni-sensitive patients to oral Ni intake. In this study, the gastrointestinal absorption of Ni was fairly similar in three of the volunteers, but was higher (+25%) for the fourth subject (S.M.). This subject is a young vegetarian woman, who may have inadequate iron intake. It has been reported that Ni absorption is increased in

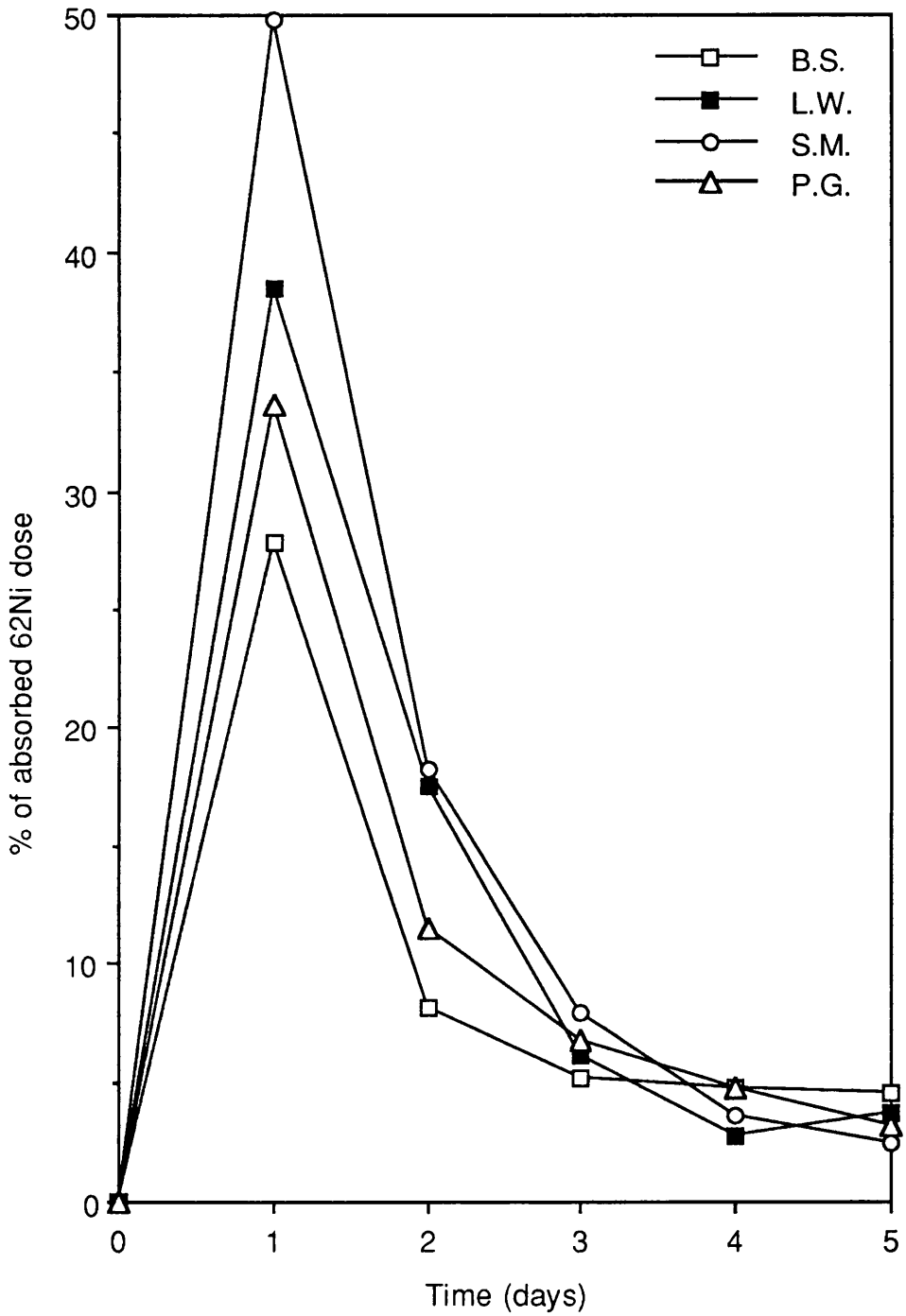

Fig. 4.7 Urinary excretion of ^{62}Ni after dosage as a percentage of the absorbed dose in the four subjects.

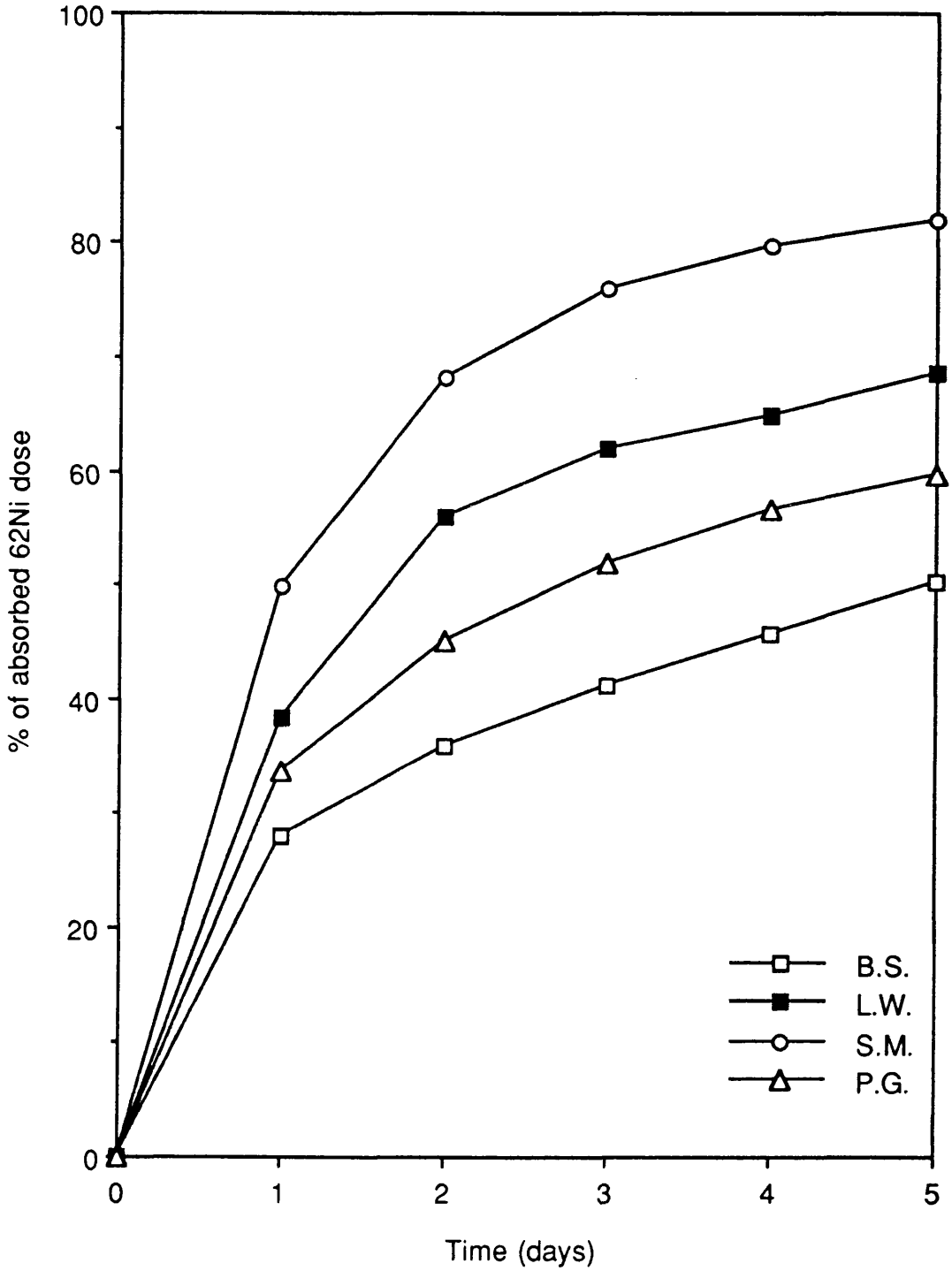

Fig. 4.8 Cumulative urinary excretion of ^{62}Ni after dosage as a percentage of the absorbed dose in the four subjects.

Table 4.10 Absorption, excretion and retention of the ^{62}Ni dose in each of the four subjects, expressed as absolute amount, percentage of the dose and percentage of the absorbed dose.

	B.S.	L.W.	S.M.	P.G.	Mean	SD
Absolute amount, µg						
Total ^{62}Ni dose, µg	670	760	571	879		
Total faecal excretion, µg	478	514	342	604		
Absorbed dose, µg	192	246	229	275		
Total urinary excretion, µg	97	169	188	166		
Retained amount, µg	95	77	41	109		
% of dose						
Total ^{62}Ni dose	100.0	100.0	100.0	100.0		
Total faecal excretion, %	71.3	67.6	59.9	68.7	66.9	4.9
Absorbed dose, %	28.7	32.4	40.1	31.3	33.1	4.9
Total urinary excretion, %	14.5	22.3	32.9	18.9	22.1	7.8
Retained amount, %	14.2	10.1	7.2	12.4	11.0	3.0
% of absorbed dose						
Absorbed dose, %	100.0	100.0	100.0	100.0		
Total urinary excretion, %	50.4	68.5	82.0	59.8	65.2	13.4
Retained amount, %	49.6	31.5	18.0	40.2	34.8	13.4

iron-deficient rats (Tallkvist et al., 1994). This suggests that iron status should be assessed in volunteers chosen for studies of Ni absorption.

The percentage of the absorbed dose excreted in urine showed a wider variability from subject to subject, that can be partially explained in terms of total amount of urine passed. The percentage of dose retained in the body at the end of the experiment ranged from 7.2 to 14.2% of the amount of ^{62}Ni given, corresponding to 18 and 49.6% of the absorbed dose.

Biliary excretion has been suggested as a route of Ni elimination on the basis of evidence of biliary excretion in rats and rabbits (Onkelinx et al., 1973) and the measurement of Ni in human bile specimens (Rezuke et al., 1987). However, Sunderman et al. (1989) did not find evidence of enterohepatic circulation of Ni, such as secondary peaks of plasma and urinary concentrations, nor did I. In this study, for the first time, I was able to assess the pattern of faecal excretion of Ni, as the tracer could be distinguished from naturally occurring Ni ingested with food. The elimination of ^{62}Ni in faeces showed a constant decline and followed the same pattern observed for the faecal marker (Figs. 4.1-4.5). This confirms that biliary excretion of Ni is absent or very small in humans.

A comparison of the estimates of Ni absorption, excretion and retention obtained in the two complete studies on humans is reported in Table 4.11.

Table 4.11 Estimates of Ni absorption, excretion and retention, as percentage of the dose, using naturally occurring Ni or a stable isotope (^{62}Ni).

	Sunderman et al. 1989 Ni 4 days		This study 1993 ^{62}Ni 5 days	
	Mean	SD	Mean	SD
Faecal excretion, %	76	19	66.9	4.9
Urinary excretion, %	26*	14*	22.1	7.8
Total excretion, %	102	8	89.0	3.1
Absorbed fraction, %	27	17	33.1	4.9
Retained fraction, %	0		11.0	3.0

* derived from figure

For all parameters, the results of this experiment show a much lower inter-individual variability than that observed by Sunderman et al.(1989), dosing with naturally occurring Ni. There is a difference in the estimate of total Ni excretion between the two investigations, although similar results are obtained for the evaluation of gastrointestinal absorption. The average faecal excretion I found was about 9% lower than that measured by Sunderman et al. and the urinary excretion was 4% lower. Whereas Sunderman et al. concluded that Ni was completely eliminated from the body within 4 days, I found that a considerable fraction of the absorbed dose (34.8% ± 13.4) was still retained five days after ingestion. These results are confirmed by recent observations of Templeton et al. (1994), who administered an oral dose of ^{61}Ni to one volunteer and reported a cumulative urinary excretion of 27% and still increasing 96 h after the isotope ingestion.

4.4 CONCLUSIONS

In conclusion, the use of a stable isotope for the investigation of Ni metabolism in humans had several advantages, including the possibility of using more physiological dosages. I needed less stringent precautions

against contamination and the fate of the tracer could be followed for a longer period at lower concentrations. The results of this study have highlighted that Ni excretion and inter-individual variability may have been overestimated and complete elimination of Ni from the body may take longer than previously reported.

Estimates of long-term retention of the absorbed Ni fraction should be considered with caution, as the non-exchangeable or slowly exchangeable fractions can apparently be increased by any losses of Ni due to incomplete faecal and urine collections. However, in this study, the faecal marker given with the Ni dose was completely recovered from all subjects and the retention of Ni was also confirmed by the plasma and urine concentrations. These results are also consistent with recent reports of long-term Ni retention in mice, that were given amounts of Ni comparable with human dietary intake, but using a radioactive gamma emitting Ni isotope (Nielsen et al., 1993).

The investigation of the processes involved in the slow exchange of Ni in tissue will require further study. Preliminary results on the kinetics of Ni uptake from erythrocytes *in vivo* have been presented. The speciation of Ni in plasma, that could also be carried out using the stable isotope technique, could provide further insight.

CHAPTER 5

CONCLUSIONS

The aim of this work was to gather information as to risk of clinical toxicity of Ni from inadvertent exposure during various medical treatments. This required a survey of sources of exposure and the determination of Ni concentration in body fluids of potentially exposed patients.

Monitoring clinical exposure to Ni is hampered by its low concentrations in body fluids, limited sensitivity of analytical techniques and the high risk of contamination. Analytical procedures need to be carefully validated to ensure that reliable results are obtained to allow meaningful conclusions.

I assessed the performances of analytical methods, based on GFAAS with deuterium background correction and ICP-MS with stable isotope dilution, for the determination of total Ni and selected Ni isotopes in biological samples. The GFAAS method proved accurate and reliable for the study of clinical exposure to Ni, although not sensitive enough for the determination of the lower Ni concentrations in serum of unexposed people. Although in principle, ICP-MS could provide lower detection limits,

the analysis of biological samples by this technique required acid digestion and solvent extraction steps, which had reagent blank levels of about 3 µg/L, and prevented the exploitation of the full potential of this technique. I intend to carry out further work to improve the present ICP-MS method, to allow the determination of lower concentrations and reduce the time required for analysis. This will include the use of microwave pressure vessel digestion and on-line separation methods, based on chelation chromatography with immobilised iminodiacetate.

I have shown that the Ni content in human albumin solutions from recent production has dramatically decreased. Similarly, the concentrations of Ni I measured in serum of a group of haemodialysed patients are lower than previously described, although still high in comparison with the most recent estimates of serum Ni concentrations for unexposed subjects (0.14 ± 0.09 µg/L).

These results indicate that the risk of Ni toxicity during clinical treatment is minimal. However, the Ni concentrations observed in human albumin solutions are still higher than normal blood serum concentrations and could trigger allergic reactions in hypersensitive subjects. People with healthy kidneys can rapidly eliminate in urine any excess of Ni inadvertently administrated to them, but the risk of Ni retention and accumulation in tissue of patients with impaired renal function remains a

possibility. Evidence for this could be obtained by comparing the Ni concentrations in autopsy tissues from patients who suffered from chronic renal failure and those suffering accidental deaths. Periodical surveys of Ni concentrations in human albumin solutions and other products for i.v. injection are desirable and could be carried out using the methods described here.

I have shown that hypernickelaemia still occurs in patients maintained on haemodialysis, although the concentrations of Ni in dialysis fluids is now less than 0.5 µg/L. Olerud et al. (1984) demonstrated that Ni can be transferred into blood against a concentration gradient due to high affinity of Ni for plasma proteins, such as albumin. However, in this work I did not obtain conclusive evidence of Ni uptake during the dialysis cycle. The observation, already reported in literature, that serum Ni concentrations do not correlate with the length of dialysis treatment was also confirmed.

In addition to Ni uptake in blood from contaminated dialysis fluids, inefficient excretion of Ni present in diet could contribute to hypernickelaemia. Dietary intake of Ni is rather high and variable, ranging from 100 to 900 µg/day, with a gastrointestinal absorption estimated between 1 and 5%. Nickel in blood is strongly bound to proteins and its excretion in urine involves the formation of a ternary complex of Ni, albumin and L-histidine (Glennon and Sarkar 1982). This mechanism may

not be efficient during dialysis and removal of Ni from the body may be incomplete. This possibility could now be investigated using the method I have developed to follow the decline of plasma concentration of a stable Ni isotope administered by i.v. injection to patients on haemodialysis. The results could then be compared with the information I obtained on healthy people.

Most of the studies of Ni absorption, distribution and excretion were carried out on animals using high Ni doses to enhance toxic responses. The findings of such investigations provide little information on the consequences of mild exposure, such as that experienced by some patients following some medical treatments. The few studies carried out on humans suffered from methodological limitations, as ethical constraints prevented the use of radioactive Ni isotopes as tracers.

I reported here the results of the first complete investigation of Ni metabolism in humans, carried out using stable isotopes. As a result of the exclusion of confounding factors, such as sample contamination and variable dietary intake, Ni absorption and excretion could be accurately evaluated and inter-individual variability assessed. The most important conclusion of this study was that, even in healthy subjects, complete elimination of Ni requires longer than previously reported. The processes involving slow-exchange of Ni and the mechanisms of Ni excretion need

to be investigated and further work will be carried out using the stable isotope technique to obtain additional information on the distribution of labelled Ni among plasma protein fractions and in red cells as a function of time. The speciation of Ni in urine could also help clarifying the mechanism of Ni excretion.

Besides providing more accurate information on Ni metabolism in healthy subjects, the stable isotope technique could be applied to investigate a number of issues, related to clinical and environmental exposure to Ni, such as the increased gastrointestinal absorption of Ni in iron-deficient subjects and patients undergoing therapy with chelating agents. Such investigations were previously restricted to animal studies (Tallkvist et al., 1994; Tallkvist and Tjälve, 1994; Nielsen and Andersen, 1994).

Differences in gastrointestinal absorption of Ni among hypersensitive subjects have been suggested as an explanation for contradictory clinical responses to oral doses of Ni sulphate. Both a low Ni dietary intake and prolonged treatment with oral Ni have been reported to be beneficial to patients suffering from Ni contact dermatitis. In a recent report, Santucci et al. (1994) claimed that therapeutic administration of oral Ni, in doses ranging from 0.6 to 2 mg Ni for periods between 3 and 6 months, reduced gastrointestinal Ni absorption, activated renal excretion and promoted

mobilisation of Ni deposits. The stable isotope technique could be used to confirm these findings and clarify the scientific basis of the therapy.

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APPENDIX

Table A1 Urinary volume, flow, creatinine excretion and creatinine clearance in the four subjects during the five days of the experiment

	URINE								U-CREATININE							
	Volume, ml				Flow, ml/min				Excretion, mmol				Clearance, ml/min/m^2			
Subjects	BS	LW	SM	PG	BS	LW	SM	PG	BS	LW	SM	PG	BS	LW	SM	PG
Time intervals																
0-3	92	142	0	0	0.51	0.79			1.75	2.68						
3-6	87	365	579	164	0.48	2.03	1.61	0.46	2.09	2.48	2.67	5.12				
6-12	38	624	376	183	0.11	1.73	1.05	0.51	0.94	4.12	1.73	4.42				
12-24	1238	281	489	296	1.72	0.39	0.68	0.41	6.69	5.19	4.30	6.89				
0-24	1455	1412	1444	643	1.01	0.98	1.00	0.45	11.46	14.47	8.70	16.43	97	87	73	85
24-48	1413	1441	1145	910	0.98	1.00	0.80	0.63	9.75	19.75	10.77	21.02	84	121	89	108
48-72	1151	1284	1275	912	0.80	0.89	0.89	0.63	8.52	14.90	10.33	17.69	76	93	92	89
72-96	903	1862	2228	1318	0.63	1.29	1.55	0.92	9.57	8.94	9.36	20.69	83	55	85	108
96-120	940	2252	2682	1292	0.65	1.56	1.86	0.90	11.84	16.67	10.19	18.61	106	102	84	112
MEAN	1172	1650	1755	1015	0.81	1.15	1.22	0.71	10.22	14.94	9.87	18.89	89	92	84	100
SD	230	358	597	257	0.16	0.25	0.41	0.18	1.24	3.53	0.74	1.75	11	22	6	11
RSD%	20	22	34	25	20	22	34	25	12	24	7.5	9.3	12.0	23.5	7.6	11.0

Table A2 Serum creatinine values (µmol/L) measured in samples from the 4 volunteers taken at the defined times.

Time (h)	BS	LW	SM	PG
0	82	102	85	111
0.5	87	98	85	109
1.5	83	100	90	111
2	82	97	84	111
2.5	84	94	80	114
6	82	103		
6.5			61	120
24	82	97	87	114
48	79	95	80	116
72	81	96	79	112
96	79	97	87	97
MEAN	82.1	97.9	81.8	111.5
SD	2.3	2.9	8.1	6.0