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Review

Reversed-phase and ion-pair separations of nucleotides, nucleosides and nucleobases: analysis of biological samples in health and disease

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ABSTRACT

Methods for the assay of nucleotides, nucleosides and nucleobases in biological samples in health and disease are reviewed, with emphasis on reversed-phase and ion-pair reversed-phase techniques for their determination. Modes of extraction from biological samples are discussed with respect of the determination of *in vivo* concentrations. Advantages and limitations of ion-pair reversed-phase chromatography are discussed with examples from biochemistry and clinical chemistry. The capacity of the high-performance capillary electrophoresis is compared with that of ion-pair reversed-phase chromatography.

CONTENTS

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LIST OF ABBREVIATIONS

CE	Capillary electrophoresis
CZE	Capillary zone electrophoresis
HPLC	High-performance liquid chromatography
HPCE	High-performance capillary electrophoresis
IP-RPC	Ion-pair reversed-phase chromatography
MS	Mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
PCA	Perchloric acid
TCA	Trichloroacetic acid
TFA	Trifluoroacetic acid

1. INTRODUCTION

The investigation of purine metabolism started with the measurement of urate. The availability of analytical-reagent grade ion-exchange resins opened up the application of liquid column chromatography to nucleotide analysis. Currently anion-exchange, reversed-phase, ion pair [1] and zwitterion reversed-phase [2] methods are available for the separation of nucleotides. More recently the separation efficiency has been substantially increased by using capillary zone electrophoresis (CZE). High-performance liquid chromatography (HPLC) is the method of choice for the determination of nucleotides.

Ion-pair reversed-phase liquid chromatography (IP-RPC) is a well established method for the separation of ionized organic compounds and inorganic ions. It allows the control of the retention of an ionic solute by modification of the concentration of the organic modifier, the ion-pair reagent, the counter ion and the pH of the eluent. Different model mechanisms of IP-RPC have been used to explain the retention and separation of polar compounds. With respect to nucleotide analysis, IP-RPC offers the possibility of the separation of polar and apolar compounds within one run and therefore represents a tool for the investigation of nucleotide metabolism in cells and tissues.

1.1. Nucleotides in metabolism

Nucleotides participate in most biochemical processes as activated precursors for DNA and RNA synthesis, as intermediate products in many biosyntheses (*e.g.*, CDP-diacylglycerine, S-adenosylmethionine), ATP as a ubiquitous source of energy in the cells, GTP and its participation in the movement of macromolecules, the translocation of newly formed peptides at ribosomes and the activation of signal coupling proteins. Adenine nucleotides represent elements of the coenzymes NAD, FAD and CoA. Cyclic nucleotides such as cAMP mediate the action of hormones. ATP modifies the activity of several enzymes by covalent modification (*e.g.*, adenylation of glutamine synthetase) and the inhibitors of nucleotide biosynthesis act as cancerostats (methotrexate folate analogues) or antiviral substances (*e.g.*, reverse transcription is inhibited by azidothymidine) [3].

Fig. 1 outlines the nucleotide pathways with their interrelationships and the regulatory action of the enzymes. Ribonucleosides and bases are freely exchanged across the cell wall. This rapid exchange with the surrounding medium affects the determination of intracellular concentrations. The turnover in the nucleotide pool is high, and therefore changes in the nucleotide pool occur very rapidly. Dissected tissue has to be frozen immediately (to inactivate the enzymes in order to obtain *in vivo* concentrations). Owing to its role in energy metabolism of the cells, ATP is quantitatively the most important compound. In experiments on the energy depletion of cells [4,5], ATP is rapidly degraded to hypoxanthine. Owing to the compartmentation of the nucleotide pool [6] inside the cells (*e.g.*, mitochondria), fractionation of these compartments has to be performed first if nucleotide levels are to be determined. Not all of these compounds (*cf.*, Fig. 1) are to be found in each cell type, *e.g.*, dGTP and dATP are not detectable in erythrocytes.

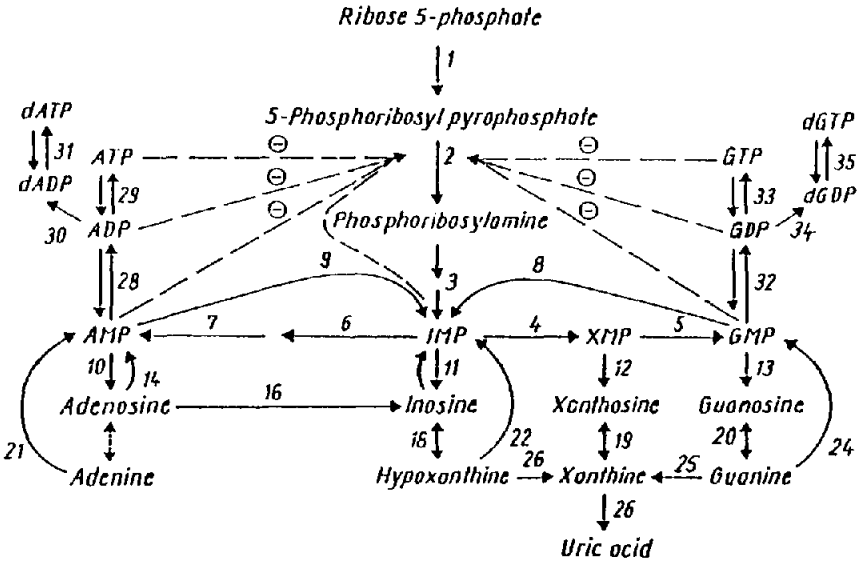

Fig. 1. Pathways of purine metabolism and possible interconversions between the different nucleotide metabolites (including negative feedback control on the enzymes of the purine *de novo* synthesis). Nucleosides and nucleobases are freely exchanged across the cell wall. 1 = Ribose-P-pyrophosphokinase; 2 = transketolase; 3 = *de novo* purine synthesis; 4 = IMP dehydrogenase; 5 = GMP synthase; 6 = adenylosuccinate synthase; 7 = adenylosuccinate lyase; 8 = GMP deaminase; 9 = AMP deaminase; 10, 11, 12, 13 = 5'-nucleotidase; 14 = adenosine kinase; 16 = adenosine deaminase; 18, 19, 20 = purine nucleoside phosphorylase; 21 = adenine phosphoribosyltransferase; 22, 24 = hypoxanthine guanine phosphoribosyltransferase; 25 = guanine deaminase; 26 = xanthine oxidase; 28 = adenylate kinase; 29, 31, 33, 35 = nucleoside diphosphate kinase; 32 = guanylate kinase; 30, 34 = ribonucleoside diphosphate reductase. (Modified after ref. 5.)

HPLC determinations of nucleotides have been applied to the investigation of errors in nucleotide metabolism (*e.g.*, severe immunodeficiency) and of energy metabolism, in oncology and in studies of antitumour metabolites, haematology (*e.g.*, blood preservation), studies of enzyme activities and monitoring of ischaemic damage.

1.2. Structural properties of nucleobases, nucleosides and nucleotides

The influence of the apolar purine and polar groups on the separation process have been widely investigated [7]. Depending on the purpose, one has to decide whether to choose reversed-phase separation (mainly for the determination of nucleosides and nucleobases), anion-exchange separation (for nucleotides based on interaction with the phosphate group) or IP-RPC for the separation of nucleobases, nucleosides and nucleotides within one run. Fig. 2 shows the structures of hypoxanthine, adenosine and guanosine monophosphate and a UV spectrum of ATP as an example.

The pK values of the nucleobases are outside the operating range of silica (pH 2–7), except for adenine (4.15), and therefore the influence of pH variations on the separation is minimal. The nucleotides are charged in this operating range of silica columns owing to their phosphate ester linkages. The nucleobases have a strong chromophore absorbing between 240 and 270 nm (*cf.*, UV spectrum for ATP in Fig. 2). Spectra of other nucleic acid compounds have been published [8]. The ratio of the absorbances at 254 and 280 nm is usually used for the identification of a substance. In samples originating from biological fluids, peak identification could be performed by using a photodiode-array detector [9,10]. For the exact validation of the peak assignment, the enzymatic peak shift method is to be preferred (involving reaction of the fractionated peak with a pure en-

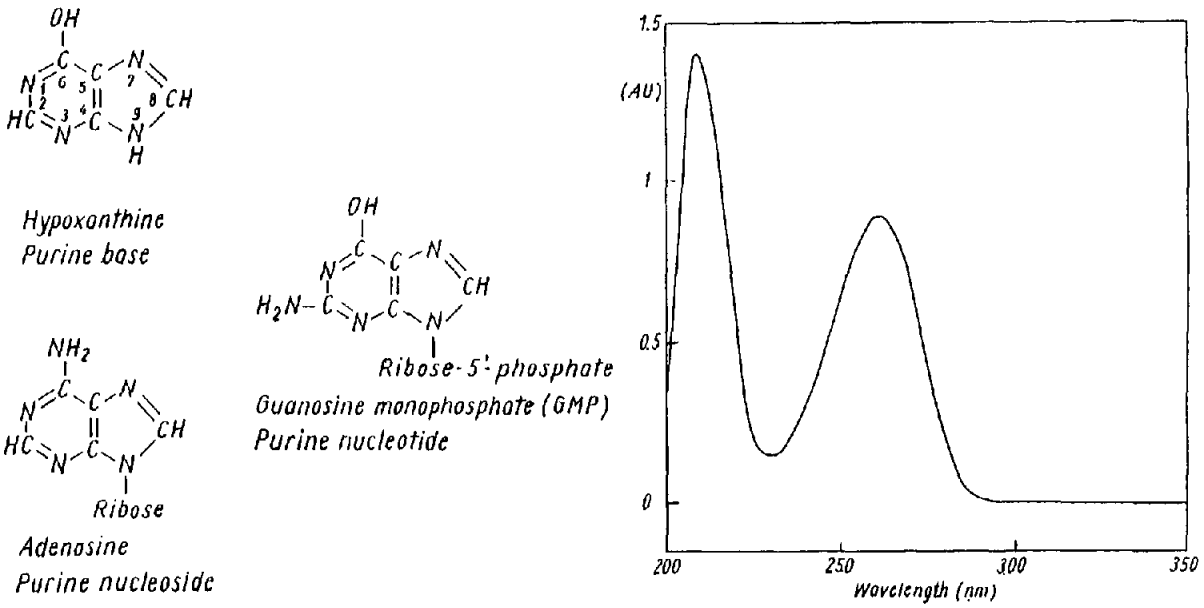

Fig. 2. Structures of hypoxanthine, adenosine and GMP and UV spectrum of ATP.

zyme, *e.g.*, 5′-nucleotidase, and reanalysis by HPLC).

The electrochemical activity of nucleotides [11,12] is highest in the pH range 7–9 [13] and therefore restricts the use of this detection principle at the common chromatographic methods. In addition to UV detection, fluorescence methods have been applied to the detection of, *e.g.*, S-adenosylmethionine [14], fluorouracil [15], thioguanine [16] and compounds after pre- or post-column derivatization [17–20]. At the low nanogram level, purine derivatives are detectable by application of mass spectrometry (MS), but this approach is limited mainly by the large amount of buffer ions in the eluent [21–23]. For most applications UV detection is the method of choice in HPLC. Fluorescence detection after derivatization could enhance the detection limit in capillary electrophoresis (CE). Fluorescence detection is also advantageous for monitoring nucleobases [17–20].

2. METHODS FOR THE DETERMINATION OF NUCLEOTIDES IN CELLS AND TISSUES

Enzyme assay, bioluminescence, ^{31}P nuclear magnetic resonance spectroscopy (NMR), HPLC, high-performance capillary electrophoresis (HPCE) and gas chromatography (GC) can be applied to the determination of nucleotides. ^{31}P NMR yields signals representing the sum of all nucleotides with similar chemical shifts. It has a great potential for the *in vivo* monitoring of relative changes in nucleotide pools. HPCE [24–26] and capillary GC [27] also allow the separation of nucleotides, nucleosides and nucleobases.

2.1. Nucleotide determination by HPLC

For the determination of nucleosides and nucleobases in extracts from cells or biological fluids, separation on reversed-phase materials with methanol as organic modifier is the method of choice [28–34]. In connection with column-switching techniques or solid-phase extraction, this offers the opportunity of direct sample injection of, *e.g.*, plasma or urine and of monitoring certain nucleosides or nucleobases as a function of disease status.

Purine nucleotides and their derivatives can be determined by stepwise gradient elution on reversed-phase columns [35–37]. The resolution of these separations is limited owing to the poor interaction of nucleotides with apolar octadecylsilane resins. Ribo- and deoxyribonucleotides are well retained on strong and weak anion-exchange materials [38–43] and on reversed-phase materials with an ion-pair reagent added [44–51].

The separation on anion exchangers is strictly controlled by the pH of the eluent (charge of the exchanger). A disadvantage of anion-exchange separations is the use of highly concentrated eluent buffers; care has to be taken with the purification of phosphates to diminish the increase in baseline rise from UV-absorbing impurities and to ensure the necessary washing cycles for prolongation of the column lifetime.

2.2. Ion-pair reversed-phase methods

Ion-pair reagents have been applied for the enhancement of solvent extraction. Ion-pair reagents are widely used for the separation of polar compounds such as certain pharmaceuticals, amino acids, proteins and nucleic acids and for chiral separations of polar compounds.

The charge of the nucleotides permits a good interaction with cationic ion-pair reagents (*e.g.*, tetrabutylammonium phosphate). The butyl groups interact with the octadecyl groups of the column, leading to good retention. The elution order of nucleotides follows the number of charges (nucleotide monophosphates first, triphosphates last). The use of tetrabutyl ions is to be preferred to tetraethyl ions because of the prolonged retention of tetrabutyl groups [1]. The eluent buffer concentration should not be increased above *ca.* 100 m*M* owing to the competition for the ion-pair reagent [52]. Improvements in separations of nucleotides by IP-RPC could be achieved by varying the pH of the eluent within the limitations given by the use of silica-based columns (range of pH stability) [48].

IP-RPC is now the method of choice for the separation of nucleotides and their derivatives within one run. It offers a high resolution in a single run (especially important for limited sample volumes). The reproducibility and the inter-column performance are good. Compared with anion-exchange separations the equilibration time is much shorter (except for the long initial equilibration time for a new column).

Different phosphate buffers at concentrations between 10 and 100 mmol/l may be used (preference is given to lower ionic strengths). Different tetrabutylammonium ions (phosphates or sulphates) could be used (concentration between 2 and 10 mmol/l). C_{18} columns from different manufacturers are suitable. IP-RPC could also be performed with microbore columns. In this instance (flow-rate below 0.3 ml/min) C_8 columns have to be used, otherwise overloading with ion-pair reagent followed by desorption could occur. Acetonitrile is used as an organic modifier.

3. EXTRACTION METHODS

In addition to improving the resolution of chromatographic separations, extraction influences the accuracy of the nucleotide values determined (especially in cells or tissues with a high turnover). Cells or organelles have to be destroyed first and the metabolism of nucleotides has to be blocked immediately by inactivation of the enzymes.

The homogenization or sonication and protein denaturation steps should be combined and shortened. It is in these steps that the largest losses in the total levels of nucleotides occur (*e.g.*, conversion of nucleotide triphosphates to nucleotide monophosphates or hypoxanthine; inclusion of nucleotides in the protein precipitate).

The determination of the recovery is the most time-consuming process in the validation of concentration determination (the method of choice is the determination of recovery after radiolabelling of the nucleotide pool).

3.1. Cells and tissues

Extraction of nucleotides from cells or tissues can be performed with perchloric acid (PCA), trichloroacetic acid (TCA) or trifluoroacetic acid

(TFA) [8,42]. The supernatant after precipitation is neutralized with Freon when using TCA [53] or diethyl ether [54]; for PCA extracts potassium hydroxide or potassium carbonate–triethanolamine is used for neutralization. Problems that arise in the extraction process are the occurrence of peaks from the buffer salts and the irreversible loss of nucleotides with the perchlorate precipitate after neutralization. The recoveries have to be determined for each buffer system used (values for PCA and TCA extraction have been published [55,56]). Acid extractions are not suitable for the determination of reduced pyridine nucleotides and must be replaced by alkaline extraction [36,55–58]. Sample preparation from tissues should preferentially use the freeze-clamp technique, followed by destruction of the cells by homogenization or sonication. In addition to the neutralization of the samples, the degradation of the nucleotides during the sample preparation has to be taken into account [59,60]. The determination of reference values for nucleosides and nucleobases in cells is therefore dependent on the rapid stopping of enzyme activities that metabolize nucleotides.

3.2. Biological fluids

Samples from biological fluids such as plasma or cerebrospinal fluid could be directly applied to HPLC analysis after solid-phase extraction (*e.g.*, on octadecylsilica columns for the removal of proteins from the sample; this process can be automated by column switching [61–63]).

Sample preparation for nucleoside and base determination should preferably be carried out using column extraction (Sep-Pak cartridge) or a micropartition filtration system (centrifugation through a deproteinization membrane) with respect to low concentration ranges and achieving optimum recoveries.

4. ION-PAIR REVERSED-PHASE CHROMATOGRAPHY: SEPARATION MECHANISMS AND APPLICATIONS

4.1. Retention mechanism

Ion-pair chromatography could be explained by the retention of charged or ionizable molecules as ion pairs on reversed-phase columns. The existence of a dynamic ion exchanger where the counter-ion moieties are bound adsorptively to the reversed-phase material has also been discussed [64]. Hydrophobic interactions with the reversed phase are decreased by increasing the amount of organic modifier, and the dielectric constant of the medium is decreased [65].

The following models for the description of the retention mechanism by IP-RPC have been developed: dynamic exchanger model; dynamic complex exchange model; ion-pair formation; and non-stoichiometric model [65,66]

For the common IP-RPC separation a mixed mode of ion-pair formation and dynamic exchange is likely to operate with gradient elution. The dynamic complex exchange model [67] describes the mechanism of the retention of nucleotides in the best way. This includes the primary layer of adsorbed ions on the reversed-phase surface by hydrophobic interaction and the attraction of oppositely charged ions. The effects on neutral samples could not be explained by this model. The ion distribution is influenced by polar or non-polar interactions depending on the salt concentration.

The resolution in IP-RPC is influenced directly by the degree of end-capping due to the interaction between residual silanol groups and amines [66]. The resolution could be increased by increasing the column length [68]. Preference is given to 250 mm × 4.6 mm I.D. columns. Run times between 40 and 55 min are possible with analytical columns of 250 mm length and a 5-μm particle size, allowing the separation of nucleobases, nucleosides and nucleotides in a single run. The application of IP-RPC to microbore columns is difficult owing to the occurrence of sorption and desorption processes.

Details of the applied methods are given in the captions of the figures.

4.2. Applications in biochemistry (nucleotide determination in cells: red blood cells, Ehrlich ascites tumour cells, hepatocytes)

TCA and PCA extraction is the method of choice for the preparation of samples from cells.

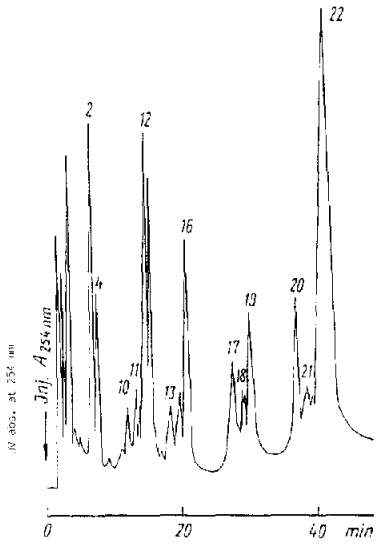

Fig. 3. Chromatogram of an acidic extract of reticulocyte-rich red blood cell mixture. Ion-pair reversed-phase separation on a Supelcosil C_{18} DB column (150 mm × 4.6 mm I.D.) with a gradient of buffer A (10 m*M* $NH_4H_2PO_4$–2 m*M* *tert.*-butylammonium phosphate) and B (A containing 20% acetonitrile); linear gradient in 5 min to 70%B, 30-min convex gradient to 100%B at a flow rate of 1 ml/min. Peaks: 2 = hypoxanthine; 4 = xanthine; 7 = uric acid; 10 = adenine; 11 = adenosine; 12 = NAD; 13 = IMP; 16 = AMP; 17 = GDP; 18 = UDP; 19 = ADP; 20 = GTP; 21 = UTP; 22 = ATP.

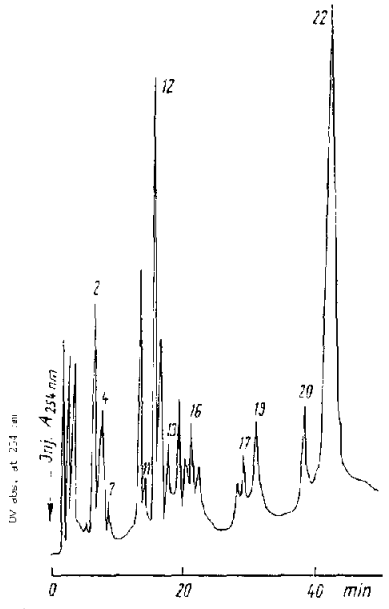

Fig. 4. Chromatogram of an acidic extract of red blood cells. Ion-pair reversed-phase separation on a Supelcosil C_{18} DB column. Chromatographic conditions as in Fig. 3.

Depending on the stage of metabolic complexity, the most appropriate separation method has to be used (*e.g.*, difference in the metabolite pattern of different cell stages of red blood cells during maturation, which is accompanied by the loss of organelles and energy-requiring processes and the switch from aerobic to anaerobic ATP production and the existence of the *de novo* synthesis of purine nucleotides). These maturation-dependent differences in the enzyme pattern lead to the interruption of the *de novo* synthesis. The extracts of erythrocytes yield a simplified peak pattern compared with reticulocytes (Figs. 3 and 4) [69–71]. Determination of nucleotide concentrations in different stages during the maturation process necessitates the enrichment of a particular stage. For this enrichment process the recovery values have to be calculated separately.

During proliferation of tumour cells (*e.g.*, cultivated hepatoma cells and Ehrlich ascites tumour cells), differences in nucleotide concentrations could also be determined by IP-RPC [72,73]. In addition to the gradient separation of nucleotides as shown in Fig. 5. for an extract of Ehrlich ascites tumour cells (with the separation of nucleobases, nucleosides and nucleotides in a single run), isocratic separations within 20 min have been used in the determination of nucleotide

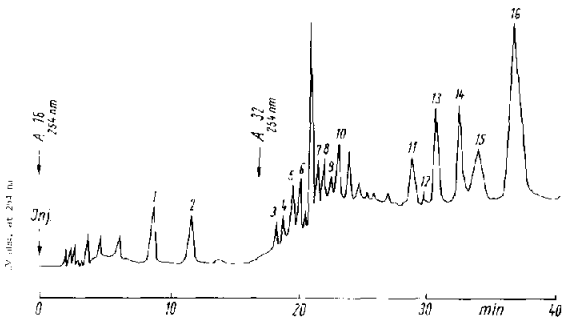

Fig. 5. Chromatogram of an extract of Ehrlich ascites tumour cells obtained by gradient IP-RPC on a Supelcosil C_{18} column (250 mm × 4.6 mm I.D.) at a flow-rate 1.3 ml/min. Buffers A and B as in Fig. 3; 12-min concave gradient to 80%B, 25 min at 80%B 20% A, 2-min linear gradient to 100% A. Peaks: 1 = hypoxanthine; 2 = xanthine; 3 = inosine; 4 = guanosine; 5 = adenine; 6 = adenosine; 7 = IMP; 8 = GMP; 9 = UMP; 10 = AMP; 11 = GDP; 12 = UDP; 13 = ADP; 14 = GTP; 15 = UTP; 16 = ATP.

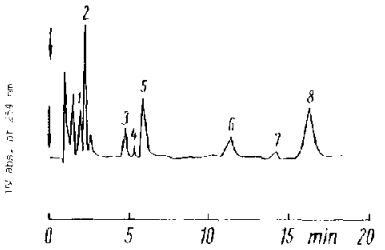

Fig. 6. Chromatogram of an extract of Ehrlich ascites tumour cells. Isocratic separation on a Nova Pak C_{18} cartridge (100 mm × 8 mm I.D.) (Waters) at a flow-rate of 2.5 ml/min with 10 m*M* $NH_4H_2PO_4$–2 m*M* *tert.*-butylammonium phosphate–15% acetonitrile. Peaks: 1 = IMP; 2 = AMP; 3 = GDP; 4 = UDP; 5 = ADP; 6 = GTP; 7 = UTP; 8 = ATP.

concentrations. For example, separations were performed at 2.5 ml/min on a Waters Radial Pak cartridge (Fig. 6). Other isocratic IP-RPC have been described [51,74].

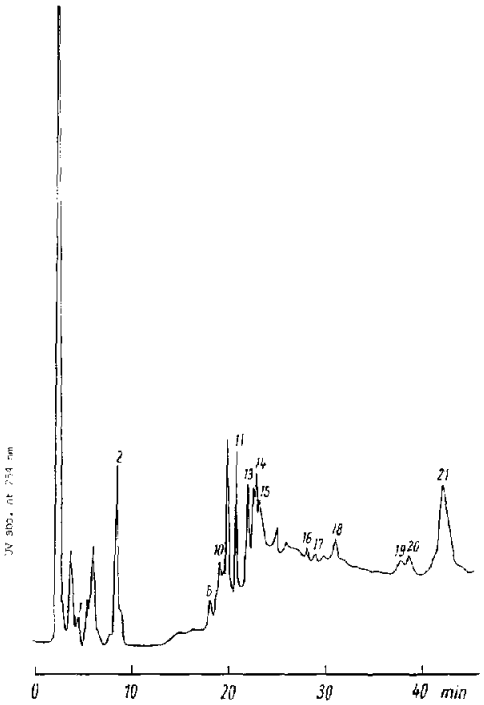

Fig. 7. Chromatogram of an extract of hepatocytes after reoxygenation. Chromatographic conditions as in Fig. 5, except for the use of a C_{18}Sil-X-5 column (250 mm × 4.6 mm I.D.) and a 25 mm × 4.6 mm I.D. guard column at a flow-rate of 1.3 ml/min and an equilibration time of 15 min. Peaks: 1 = uracil; 2 = hypoxanthine; 8 = guanosine; 10 = adenosine; 11 = IMP; 13 = GMP; 14 = UMP; 15 = AMP; 16 = GDP; 17 = UDP; 18 = ADP; 19 = GTP; 20 = UTP; 21 = ATP.

4.3. Ion-pair reversed-phase chromatography for the determination of nucleotides during ischaemia

The nucleotides in cells subjected to ischaemia undergo a very rapid breakdown which is monitored by HPLC analysis of extracts of these cells (*e.g.*, hepatocytes, Fig. 7 [55]). After reoxygenation of these cells, regeneration of the nucleotide pool occurs depending on the previous time of anoxia [75].

The same methods could be applied for monitoring of changes during the ischaemia of tissues (*e.g.*, rat small intestine, Fig. 8 [76]). The tissue samples have to be prepared using liquid nitrogen-cooled tongs, stored in liquid nitrogen until extraction and then homogenized in PCA with subsequent neutralization. For this extraction procedure the recoveries have to be determined with respect to the time of the overall extraction process. In addition to IP-RPC, anion-exchange separation could also be applied in nucleotide determinations [77], but it is impossible to determine nucleosides and nucleobases in the same run. Therefore, the preferred method for tissue analysis during ischaemia (where the sample vol-

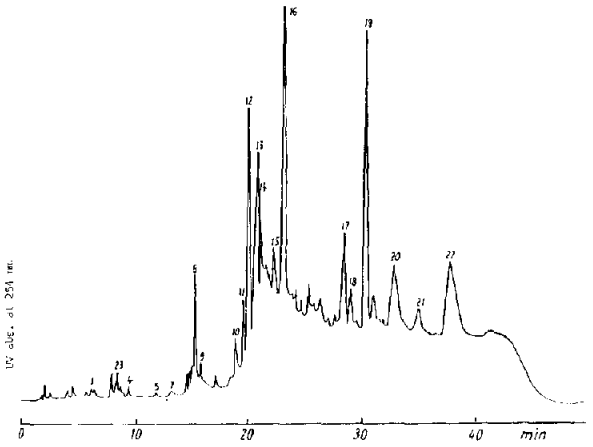

Fig. 8. Chromatogram of an extract of rat small intestine. Chromatographic conditions as in Fig. 5, except for the use of a C_{18}Sil-X-5 column (250 mm × 4.6 mm I.D.) at a flow-rate of 1.3 ml/min. Peaks: 1 = uracil; 2 = hypoxanthine; 3 = uridine; 4 = xanthine; 5 = oxypurinol; 7 = uric acid; 8 = inosine; 9 = guanosine; 10 = adenine; 11 = adenosine; 12 = NAD; 13 = IMP; 14 = GMP; 15 = UMP; 16 = AMP; 17 = GDP; 18 = UDP; 19 = ADP; 20 = GTP; 21 = UTP; 22 = ATP.

ume is limited) is IP-RPC. The drastic alterations in the metabolism of cells and tissues that occur during ischaemia–reoxygenation (*e.g.*, myocardial infarction and intestinal inflammation) are monitored in samples of these cells by IP-RPC and allow investigations of the sequence of degradation processes in nucleotide metabolism (decrease in nucleoside triphosphates and increase in nucleoside monophosphates, followed by accumulation of nucleosides and nucleobases).

4.4. Determination of reference values for disease markers (hypoxia and tumour markers, drug monitoring)

In clinical chemistry, HPLC can be used for the analysis of hypoxia [7] and tumour markers [33,78–80]. Hypoxanthine, xanthine and uridine determined in extracts from body fluids can provide information about ATP depletion in cells or organs [7]. Fig. 9 demonstrates the accumulation of hypoxanthine in a sample of rat small intestine after ischaemia. Extracts of cerebrospinal fluid, amniotic fluid, urine and plasma are mainly analysed by reversed-phase methods to determine the accumulation of these hypoxia markers. IP-RPC methods are applied if some particular drug metabolites after drug therapy have to be determined [81].

The determination of nucleosides in urine, serum and plasma shows noticeable differences between healthy subjects and individuals with various types of cancer (elevation of guanosine and inosine levels, presence of N^2, N^2-dimethylguanosine and increased concentrations of N^2-methylguanosine and 1-methylinosine in the serum of patients with breast and lung cancer and lymphocytic leukaemia). 1-Methylguanosine and methylinosine become elevated in the urine of patients with lung, colon, breast and other types of cancer. Reversed-phase HPLC analysis may yield some hints about the existence of cancer [78,79]. It has been reported that patients with malignant diseases showed consistent elevations of modified nucleosides and these were highest in patients with more advanced disease, demonstrating the potential value of modified nucleosides as cancer biomarkers [82].

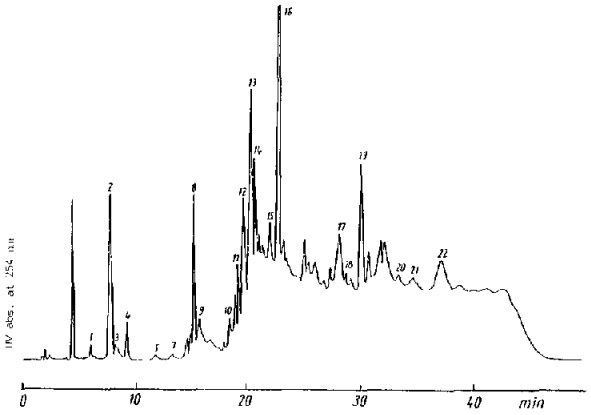

Fig. 9. Hypoxanthine (peak 2) accumulation in rat small intestine after ischaemia. Chromatographic conditions as in Fig. 8.

The exact measurement of drug and metabolite concentrations is necessary during the application of antileukaemic and antiviral drugs in order to minimize the damage to other cells and to estimate the metabolic conversion [5,49,63,83–87]. IP-RPC has been used for the investigation of disorders of nucleotide metabolism [11,86,88].

5. HIGH-PERFORMANCE CAPILLARY ELECTROPHORETIC SEPARATION OF NUCLEOTIDES

The resolution of nucleotides, nucleosides and nucleobases by IP-RPC has been increased by the use of HPCE. HPCE allows very rapid separations of nucleobases (within 7 min [24]) and better resolved separations of nucleosides (within 35 min [24]), which were obtained with a buffer system containing sodium tetraborate and sodium dodecyl sulphate. An example of the separation of nucleotide mono- and diphosphates is given in Fig. 10. Recently, concentrations of purine compounds in serum from newborns determined by HPCE were published [89].

HPCE seems to be a promising approach for the investigation of nucleotide metabolism. With respect to analysis time, HPCE could become superior to IP-RPC. At present the main disadvantage is the higher detection limit of HPCE, which necessitates a preconcentration step to allow the concentration of a large sample amount to a smaller volume. The need for maintenance of columns in HPLC could be diminished when using fused-silica capillary columns in HPCE.

6. CONCLUSION

The IP-RPC determination of nucleotides and their derivatives has the advantage over other HPLC methods that ionized and non-ionized compounds with their divergent properties can be resolved within a single run, and therefore the determination of bases and nucleotides in one sample is possible (especially with a limited sample volume).

The disadvantages are mainly related to the diminished lifetime of the columns used (irreversible adsorption of the ion-pairing molecule on the column [90]) and the long equilibration times necessary when starting IP-RPC gradient separations on a new column.

HPCE provides better resolution [24–26], but is limited by the need for a preconcentration step (to lower the detection limit) and the use of two electrophoretic separation conditions for the polar nucleotides and the apolar nucleobases and nucleosides. Future developments in the optical detection unit of HPCE systems should overcome the first disadvantage.

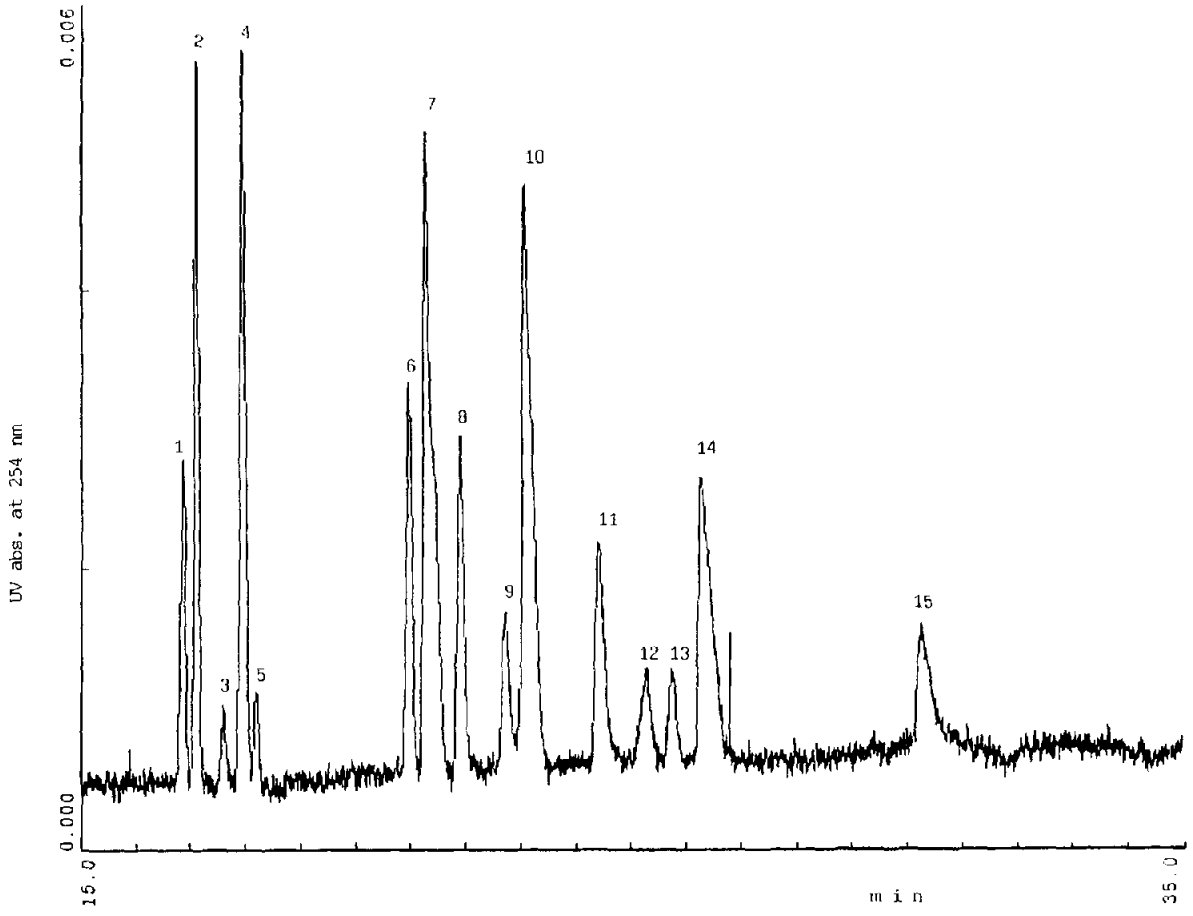

Fig. 10. Electropherogram obtained on an untreated fused-silica column (75 cm × 75 μm I.D.) with 3-(cyclohexylamino)-1-propanesulphonic acid (CAPS) buffer at pH 10.5, 15 kV. Peaks: 1 = AMP; 2 = dAMP; 3 = CMP; 4 = dCMP; 5 = dTMP; 6 = GMP; 7 = dGMP + UMP; 8 = dADP; 9 = ADP; 10 = dGDP; 11 = CDP; 12 = GDP; 13 = dCDP; 14 = dTDP; 15 = UDP.

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