Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 784 (2003) 265–274
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Method for monitoring alginate released in biological fluids by high-performance anion-exchange chromatography with pulsed amperometric detection

Mireille Desille[a], Nathalie Allain[a], Jean-Pierre Anger[a], Stephan Mahler[a], Vincent Lognoné[b], Yannick Mallédant[a,c], Bruno Clément[a,*]

[a]*INSERM U456—Détoxication et Réparation Tissulaire, Université de Rennes I, Rennes, France*
[b]*Centre d'Etude et de Valorisation des Algues (C.E.V.A.), Pleubian, France*
[c]*Département de Réanimation Chirurgicale, Hôpital Pontchaillou, Rennes, France*

Received 2 July 2002; received in revised form 7 October 2002; accepted 8 October 2002

Abstract

The use of alginate-entrapped cells in cell therapy requires a method for monitoring possible released compound within biological fluids following either their implantation or inoculation in artificial organs. Oligomannuronic and oligoguluronic acids were prepared by enzymatic depolymerization with alginate lyase from *Pseudomonas alginovora*, characterized by high-performance anion-exchange chromatography with pulsed amperometric detection and quantitated in human, pig and rabbit blood, urine and tissue samples. The method was tested for linearity and detection limit, accuracy, intra- and inter-day precision. The limit of detection was 3 μg/ml in both urine and plasma and 5 mg/g of tissues. The relative standard deviations (RSDs) of intra-day precision were 6.0–16.6% and 4.8–8.7% in plasma and urine, respectively; the RSDs of inter-day precision were 5.1–14.4% and 5.0–11.6% in plasma and urine, respectively. Thus, this method appears suitable for the measurement of released alginate from entrapped cells used in cell therapy.

Keywords: Encapsulated cells; Pulsed amperometric detection; Alginate lyase; Uronic acids; Alginate

1. Introduction

Alginates are the salts of alginic acids, consisting of algal polysaccharides which are linear copolymers of (1–4)-linked α-L-guluronic acid (G) and (1–4)-linked β-D-mannuronic acid (M) (Fig. 1). The uronic acid residues are organized in blocks of polymannuronic acids (M–M), polyguluronic acids (G–G) and heteropolymeric sequences containing alternative structures (M–G) [1]. The relative proportion of the uronic acids is very important as it determines and confers the gel-forming and viscosifying properties of alginates. Sodium alginates are commonly used as food additives and antiacid adjuvants [2] and in a

*Corresponding author. INSERM U456—Détoxication et Réparation Tissulaire, Faculté de Pharmacie, Université de Rennes I, 2 Avenue Pr Léon Bernard, 35043 Rennes Cedex, France. Tel.: +33-2-2323-4799; fax: +33-2-2323-4794.
E-mail address: bruno.clement@univ-rennes1.fr (B. Clément).

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00801-2

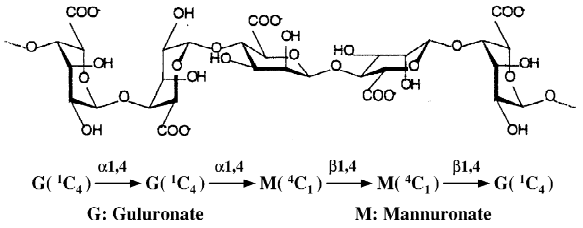

Fig. 1. Chemical structure of alginate.

number of medical applications ranging from dental implants to wound coverings and skin grafts [3–5]. Alginate gels are formed by a reaction between divalent cations such as calcium and the G residues on the alginate chain. Alginate crosslinked with Ca^{2+} has been popularized for in vitro cell culture [6,7], and tissue engineering applications [8,9], based on its ability to form hydrogels under very mild conditions [10].

One of the most exciting prospect for alginate-immobilized cells is their potential use in cell therapy, particularly for both intracorporeal implantation of differentiated cells and extracorporeal bioartificial organs. Indeed, alginate gel constitutes a three-dimensional scaffold for cells which favors the maintenance of their tissue specific functions and allows maximum mass exchanges. In addition, the alginate network may act as an immune barrier between the immobilized cells and the immune system of the host. In early works, Chang has proposed microencapsulation as a possible tool to create artificial organs [11]. Then, successful immobilization of various cells was shown with possible clinical use, including Langerhans islets for the treatment of diabetes, parathyroid cells for hypocalcemia, dopamine-producing cells for Parkinson's disease and hepatocytes for either fulminant hepatitis or metabolic genetic defects. We have shown that hepatocytes immobilized within alginate beads were viable and functional in vitro [12], and they can be used within an extracorporeal bioartificial liver to supply liver functions in animal models of enzyme deficiency and acute liver failure [13,14].

A possible shortcoming of both intracorporeal and extracorporeal use of alginate embedded cells is the potential release of alginate at the contact of biological fluids present in blood and tissues. Although alginate is known to exhibit minimal cytotoxic effects and reduced hemolysis [15–18], the clinical context requires the availability of a sensitive method for monitoring low quantities of alginate in blood, urine or tissues. Polysaccharide concentrations are not easy to measure; generally, they require a preliminary enzymatic depolymerization and/or chemical hydrolysis. Simple carbohydrates released after depolymerization, i.e., uronic acids in the case of alginate, can be detected by physical, chemical or chromatographic methods. Enzymatic depolymerization is performed with alginate lyases which display variable specificities for the homo- and heteropolymerics sequences and cleave glycosidic bonds of alginate by a β-elimination reaction mechanism [19]. High-performance anion-exchange chromatography coupled to pulsed amperometric detection (HPAEC–PAD) was shown to be a highly sensitive and specific method for carbohydrate analysis and it is a widely used technique for the chromatography of many carbohydrate-containing samples [20]. Pulsed amperometric detection enables the direct sensitive detection of carbohydrates, glycols and alditols. At pH>12, uronic acids are partially ionized and thus can be separated by anion-exchange mechanisms on a CarboPac PA-100 column. These weak acids are poorly retained by the NH_4^+ groups of the pellicular resin beads. Elution is improved by using sodium acetate gradient in sodium hydroxide. Uronic acids contain in their structure secondary hydroxyl groups (–CHOH–) which can be oxidized at a specific potential (+0.05 V). They are detected by measuring the electrical current generated by their oxidation at the surface of a gold electrode. The oxidation current is directly proportional to the concentration of uronic acids. Other products of oxidation (formates, acetates, etc.) can poison the surface of the electrode. To prevent this inconvenience, three successive potentials are applied: one to measure, one to clean up and one to recondition the electrode.

In this report, we describe the validation and application of a method for the quantitation of alginate in biological fluids. This method involves enzymatic depolymerization of the samples using *Pseudomonas alginovora* alginate lyase [19] completed by chemical hydrolysis with 1 *M* H_2SO_4, deproteinization and separation on an anion-exchange column and pulsed amperometric detection. It has selectivity without interference from biological

samples and tissues components. Detection limits, linearity and precision are reported for the CarboPac PA100 column.

2. Experimental

2.1. Chromatography apparatus

Chromatography was performed on a Dionex (Sunnyvale, CA, USA) DX-500 ion chromatograph. The system consisted of a quaternary gradient pump (GP40) and an electrochemical detector (ED40) including a detection cell with a gold working electrode and a pH-Ag/AgCl reference electrode. A personal computer equipped with the Dionex PeakNet 4.30 Window based software allowed the acquisition and processing of chromatograms and data. Table 1 summarizes the details of the operating parameters. Peak identification was based on retention time and peak spiking with authentic standard.

2.2. Chemicals

Sulfuric acid was obtained from Merck (Darmstadt, Germany); sodium acetate, sodium hydroxide and sodium chloride were purchased from Carlo Erba (Milan, Italy), Tris was from Life Technologies (New York, NY, USA), $MgCl_2$ and mannuronic acid were purchased from Sigma (St. Louis, MO, USA). Sodium alginate was from Pronova Biopolymer (Oslo, Norway). The deionized water used in the preparations of standard and sample solutions and eluents was obtained from a Milli-Q water system (Millipore, St. Quentin-en-Yvelines, France). Mannuronic acid standard solutions to be injected were prepared fresh daily by dilution of the stock solution.

2.3. Conditions of alginate depolymerization, standard and sample preparation

P. alginovora alginate lyase was prepared in the C.E.V.A. laboratory (Pleubian, France) as described by Chavagnat et al. [19]. Enzymatic blend cleaves M–M, G–G and M–G linkages. A 10-mg amount of enzyme was dissolved in 2 ml of buffer (20 m*M* Tris, 2 *M* NaCl, pH 7.5). A 1-ml volume of experimental buffer (40 m*M* Tris, 13.3 m*M* $MgCl_2$, pH 7.5) containing 20 μl of alginate lyase solution was added to the sample containing alginate. Mixture was kept under slow stirring at 25 °C for 18 h. After incubation time, 2 ml of 2 *M* H_2SO_4 was added to each assay and solutions were brought to 100 °C for 2 h for chemical hydrolysis. After cooling, solutions were neutralized by addition of NaOH and volumes were adjusted to 25 ml with deionized water. A 5-ml volume of each solution were passed through one

Table 1
Parameters for the HPAEC–PAD system

Analytical column	CarboPac PA100 (250×4 mm I.D.), Dionex (Sunnyvale, CA, USA)
Guard column	CarboPac PA100 (50×4 mm I.D.), Dionex
Column temperature	30 °C
Eluent A	Plasma: 100% (80 m*M* sodium hydroxide–120 m*M* sodium acetate) Urine: 100% (100 m*M* sodium hydroxide–100 m*M* sodium acetate) Tissue: 100% (100 m*M* sodium hydroxide–140 m*M* sodium acetate)
Eluent B	100% (250 m*M* sodium hydroxide–1 *M* sodium acetate)
Gradient[a], % A+% B (time)	Plasma: 100% A (0–15 min), 100% B (15–25 min), 100% A (25–35 min) Urine: 100% A (0–15 min), 100% B (15–30 min), 100% A (30–40 min) Tissue: 100% A (0–10 min), 100% B (10–20 min), 100% A (20–30 min)
Eluent flow-rate	0.6 ml/min
Sample loop volume	100 μl
Injection volume	50 μl
Detection	PAD
Detection mode	Integrated amperometry
Detection settings, *E* (time)	0.05 V (0.40 s), 0.75 V (0.20 s), −0.15 V (0.40 s)

[a] The gradient can vary with the age of the column. The last 10 min of the gradient was equilibration before the next injection. Both eluents A and B were degassed with helium.

On-Guard-Ag and two On-Guard-H (Dionex) to eliminate chlorides prior to injection of 50 μl into the HPAEC–PAD system.

Alginate measurements were performed in rabbit, porcine and human plasma and urine, and rabbit tissues (heart, lung, spleen, liver, intestine, kidney and brain).

Enzymatic depolymerization was performed in 1 ml of plasma spiked with 25 to 150 μl of a 0.5% alginate solution (dissolved in deionized water). After enzymatic depolymerization, samples were deproteinized by heating 5 min at 100 °C and centrifugation for 5 min at 3000 *g*. The supernatant was recovered and used for chemical hydrolysis.

A 3-ml volume of urine, spiked with 60 to 600 μl of a 1% alginate solution, were desalted by dialysis in 5 l of deionized water, during 48 h at 4 °C. A 250-μl volume of dialysis urine was used for enzymatic depolymerization after addition of 750 μl of NaCl 0.9% and 1 ml of experimental buffer containing enzyme. After enzymatic depolymerization, samples were directly subjected to chemical hydrolysis.

Tissue samples (50 mg) preparation were spiked with 25 and 50 μl of a 0.5% alginate solution and then treated as followed. Tissues were lyophilized and homogenized in liquid nitrogen. A 50-mg amount of homogenate was suspended in 2 ml of experimental buffer containing 20 μl of enzyme. After enzymatic depolymerization, the mixture was deproteinized by heating 5 min at 100 °C and centrifugation for 5 min at 3000 *g*. The supernatant was recovered and subjected to chemical hydrolysis.

2.4. Method validation

The linearity between the peak areas of both mannuronic and guluronic acids versus the concentration was evaluated using the human plasma and urine standard solutions. Four human urine standard solutions were prepared in triplicate in the range of 3–20 μg/ml (3, 5, 10 and 20 μg/ml). Five human plasma standard solutions were prepared in duplicate in the range of 3–30 μg/ml (3, 5, 10, 20, and 30 μg/ml). Standard human plasma and urine samples containing alginate were analyzed as described above. The peak area was measured and a calibration curve was obtained from a linear regression of the peak area versus spiked concentrations. The regression lines were used to calculate concentrations of alginate in the unknown human plasma and urine samples based on the peak area. A stock solution of the mannuronic acid was prepared in deionized water prior the use at a concentration of 100 mg/l. Appropriate amount of this solution was added to each working standard solution to give a final concentration of 5 μg/ml of mannuronic acid.

The intra-day and inter-day precision of the method were estimated by assaying replicate human plasma and urine samples at five different concentrations, in three analytical runs. The intra- and inter-day relative standard deviations (RSDs) of the mean were used to validate the precision and accuracy of the assay by quantifying standard samples of alginate in human plasma and urine. Intra-day variability was tested with 10 replicates of each standard concentration ($n=10$). Inter-day variability was conducted over 3 days with six replicates of each standard concentration ($n=18$).

2.5. Alginate bioavailability

A single dose of 80 mg of MVG sodium alginate (Pronova Biopolymer) was dissolved in 8 ml of 0.9% NaCl and was administered intravenously in the marginal vein of the ear of five New-Zealand rabbits (4 kg) (Elevage Scientifique des Dombes, Châtillon sur Chalaronne, France). Blood and urine samples were taken at 12 time points from rabbits during 24 h. Rabbits were euthanized 24 h after injection. Tissues (liver, lung, heart, spleen, kidney, intestine, and brain) were perfused with 0.9% NaCl for blood elimination and frozen at −80 °C until analysis. Alginate levels were measured in blood, urine and tissue samples.

3. Results

3.1. Measurement of sodium alginate in biological liquids and tissues by HPAEC–PAD

Enzymatic and chemical depolymerization of alginate were performed in plasma, urine and tissues,

which lead to mannuronic and guluronic acids, which were separated by HPAEC–PAD. Typical chromatograms of human plasma and urine samples are shown in Figs. 2 and 3, respectively, and in the rabbit tissues in Fig. 4. Mannuronic and guluronic acids were detected in plasma, urine and in all tissues analyzed, i.e., kidney, liver, brain, heart, lung, spleen, and intestine, and no interference from endogenous substances was detected. The retention times of guluronic and mannuronic acids were 11

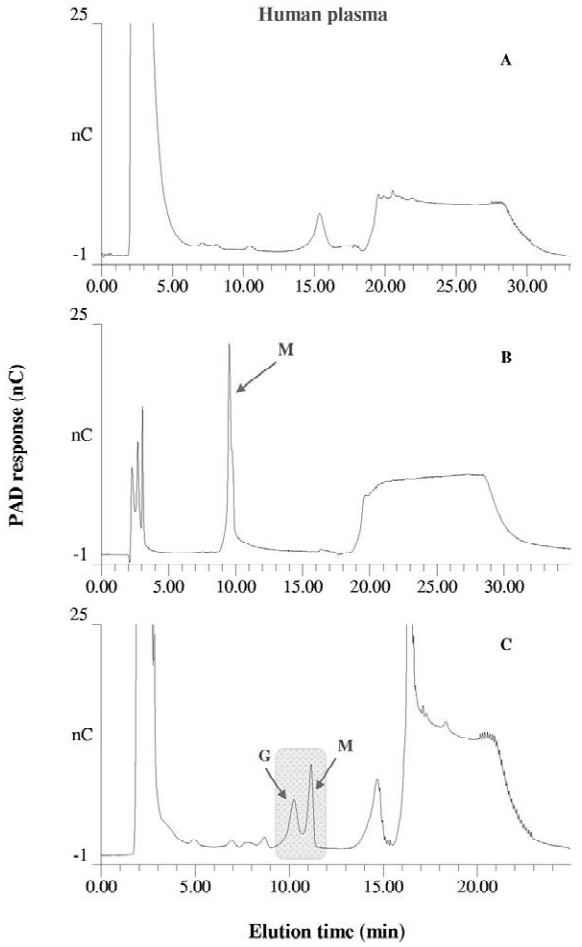

Fig. 2. Representative HPAEC–PAD chromatograms for human plasma assays. (A) Blank plasma, (B) blank plasma spiked with 5 μg/ml mannuronic acid standard, (C) blank plasma spiked with 20 μg/ml sodium alginate. Profiles are presented as response in nanoCoulomb (nC) as a function of time (min) after the cycle start. G=Guluronic acid, M=mannuronic acid.

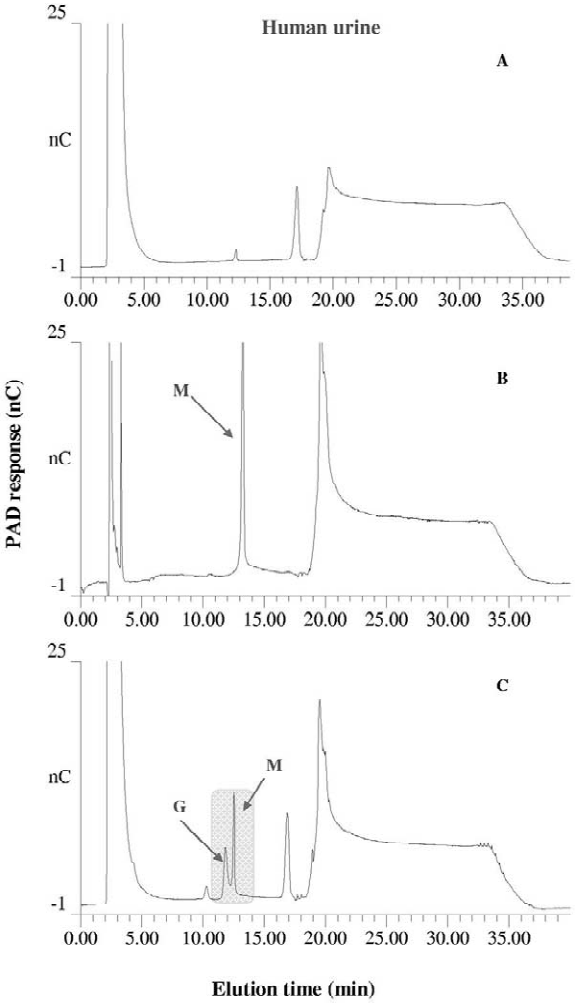

Fig. 3. Representative HPAEC–PAD chromatograms for human urine assays. (A) Blank urine, (B) blank urine spiked with 5 μg/ml mannuronic acid standard, (C) blank urine spiked with 20 μg/ml sodium alginate. Profiles are presented as response in nanoCoulombs (nC) as a function of time (min) after the cycle start. G=Guluronic acid, M=mannuronic acid.

and 13 min in plasma and urine, respectively, and 8 and 10 min in tissues, respectively. Good separation and baselines with low background were observed. The peaks of interest were well resolved. The assay methods were selective as demonstrated by the lack of interfering peaks in the blank plasma, urine and tissues samples following a visual inspection of the chromatograms.

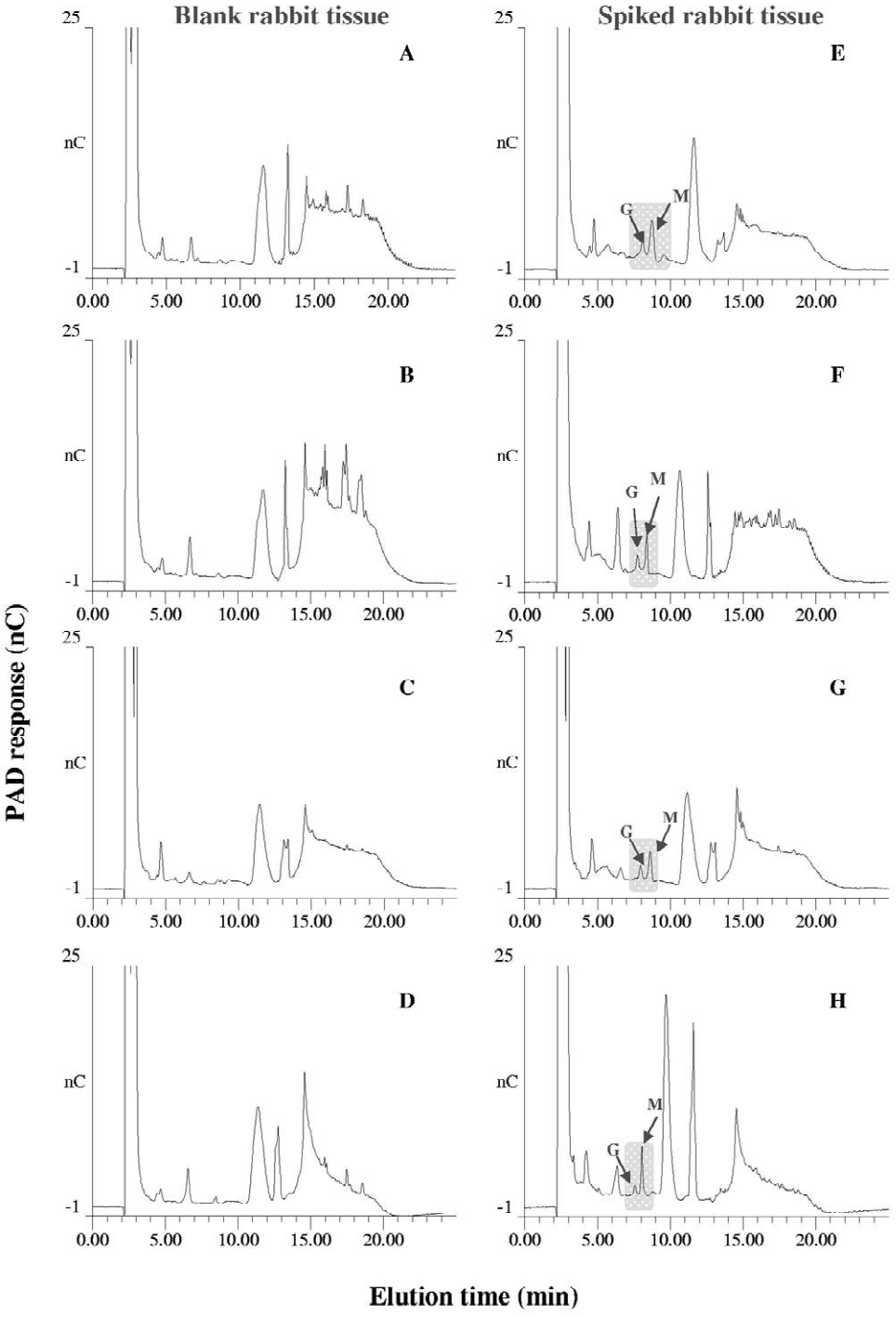

Fig. 4. Representative HPAEC–PAD chromatograms for rabbit tissues. Blank tissues (A: kidney, B: liver, C: brain, D: heart) and blank tissues spiked with 10 μg/ml sodium alginate (E: kidney, F: liver, G: brain, H: heart). Profiles are presented as response in nanoCoulombs (nC) as a function of time (min) after the cycle start. G=Guluronic acid, M=mannuronic acid.

3.2. Calibration data and limits of detection

The linearity of the method was assessed by adding known amounts of alginate to human urine and plasma over the ranges of 3 to 20 μg/ml and 3 to 30 μg/ml, respectively, prior to depolymerization. Plasma and urine standard curves were generated by weighed linear regression analysis and were found to be linear with correlation coefficients of more than 0.99. The mean slope (peak area) was 3576 with a standard deviation (SD) of 148 and an RSD of 4.15% in urine, and the mean slope was 4310 with an SD of 483 and an RSD of 11.22% in plasma. Limits of detection evaluated with a signal-to-noise ratio of three were near 3 μg/ml in both urine and plasma and 5 mg/g in tissue.

3.3. Precision and accuracy

The method was validated with respect to the repeatability (within-day precision) and internal reproducibility (day-to-day precision). Table 2 shows the repeatability calculated for 10 injections of four and five different concentrations of alginate ($n=10$) in human urine and plasma, respectively, and the internal reproducibility during analysis of different concentrations of alginate. The samples were analyzed after six injections of each standard concentration on 3 different days ($n=18$). The intra-day RSDs were 6.0–16.6% and 4.8–8.7% in plasma and urine (Table 2), respectively, and the inter-day RSDs were 5.1–14.4% and 5.0–11.6% in plasma and urine (Table 2), respectively, indicating a good precision of the analytical method. These results were assessed based on acceptance criteria of not more than 15% RSD for precision.

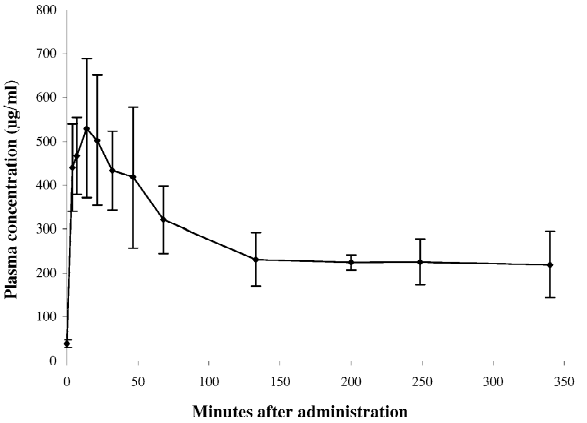

Fig. 5. Plasma alginate concentration–time profile in five rabbits following an intravenous bolus injection of 20 mg/kg alginate. Blood samples were taken at 12 time points from rabbits during 24 h. Results are the means±SD ($n=5$).

3.4. Alginate bioavailability in rabbits

Our HPAEC–PAD method was successfully applied to a pharmacokinetic study after intravenous administration of alginate to rabbits. Fig. 5 shows the

Table 2
Intra-day and inter-day precision of the HPAEC–PAD method for mannuronic and guluronic acids in human plasma and urine samples

Concentration of sodium alginate added (μg/ml)	Intra-day[a]		Inter-day[b]	
	Measured value (μg/ml) (mean±SD)	Precision (RSD, %)	Measured value (μg/ml) (mean±SD)	Precision (RSD, %)
Plasma				
3	2.60±0.38	14.61	2.71±0.39	14.39
5	3.85±0.23	5.97	3.85±0.50	5.08
10	11.83±0.78	6.59	12.47±1.10	8.82
20	18.26±1.54	8.43	20.11±1.21	6.02
30	31.79±5.28	16.61	32.63±1.82	5.58
Urine				
3	2.00±0.12	6.00	1.99±0.10	5.02
5	4.35±0.21	4.83	4.50±0.52	11.56
10	14.42±1.13	7.84	14.23±1.07	7.52
20	20.89±1.81	8.66	21.34±1.53	7.17

[a] Ten samples per concentration.
[b] Eighteen samples per concentration.

representative plasma concentration–time profile following an intravenous bolus injection of 20 mg/kg of alginate to five rabbits. The absorption reaches a maximum after 19.0±2.5 min. Thereafter, the plasma concentration declines with an apparent half-life of about 108.7±24.3 min. The elimination following intravenous administration may occur in a biphasic mode, thus suggesting a two-compartment model. Monomers of alginate molecules, i.e., mannuronic and guluronic acids, remained detectable only in blood samples. They were not detected in the urine and tissue analyzed.

4. Discussion

The technique of choice for measuring carbohydrates is HPAEC–PAD [20]. It allows a direct quantification of non derivatized carbohydrates at low picomole levels with a minimal sample preparation and clean-up. High-performance anion-exchange chromatography with pulsed amperometric detection takes advantage of the weakly acidic nature of carbohydrates to give highly selective separation at high pH values using a strong anion-exchange stationary phase. HPAEC–PAD has been widely used for analysis of mono-, oligo- and polysaccharides.

In the present study we show that the HPAEC of uronic acids with alkaline eluent systems provides a powerful tool in the analysis of alginate in biological fluids. The combination of this chromatographic system with PAD provided a highly selective and sensitive method well suited to complex samples like blood and tissues and the CarboPac PA100 column allowed an appropriate separation of mannuronic and guluronic acids in all the tested biological samples. The linearity, repeatability, precision and accuracy of the method were determined with satisfactory results.

Uronic acids were analyzed by HPAEC–PAD at a programmed column temperature of 30 °C. Oligosaccharides showed decreased retention times with increased temperature [21]. Even small differences in temperature, i.e., ±5 °C resulted in changes in retention times, indicating that standard room temperature is not satisfactory for HPAEC of oligosaccharides.

The most commonly used method for monosaccharide analysis is high-performance liquid chromatography (HPLC). Various precolumn derivatization HPLC methods with UV chromophore or fluorophore reagents such as 2-amino-pyridine [22], 1-phenyl-3-methyl-5-pyrazolone [23], 2-aminobenzoic acid [24], and 4-aminobenzoic ethyl ester [25] have been employed for the analysis of monosaccharides. Gas chromatography was used to resolve many small saccharides which have undergone derivatization. However, it is not directly amenable to larger oligosaccharides or polysaccharides. Polysaccharides must be hydrolyzed and the released monosaccharides are oxidized and converted in volatile compounds. The derivatization step is necessary in order to increase the volatility of the original saccharide molecule. These methods give high sensitivity and resolution. However, complicated derivatization procedures are necessary. Capillary electrophoresis is a powerful separation technique for monosaccharide analysis of glycoproteins [23,26,27]. Since carbohydrates lack both a charge and a strong UV chromophore, several derivatization techniques [23,26,27] have been carried out. While the derivatization method yields higher sensitivity [23], the complexity of this derivatization limits its use. HPAEC–PAD is useful for the analysis of underivatized uronic acids [28]. It minimizes the sample preparation and it is easy and sensitive by providing efficient separation and detection.

Since the high concentrations of ions modify the elution time, samples were diluted and consequently would not permit to reach a sufficient sensitivity. Chlorides came from the enzymatic depolymerization step and were eliminated from injection samples after solutions passed through Ag- and H-cartridges. Sulfates came from the chemical hydrolysis and they can be eliminated by precipitation with barium salts or barium hydroxide but new ions appear in the solvent phase that results in disturbances in elution of uronic acids. So, they modify the elution time. Since samples were not diluted, sulfates and uronic acids were quickly eluted and not separated. We have diluted samples in deionized water after chemical hydrolysis and prior to the injection. Time elution of uronic acids was longer and they could be separated from peak corresponding to sulfates.

Enzymatic depolymerization uses alginate lyases which are found in a variety of organisms including marine molluscs, echinoderms, bacteria and fungi.

The enzyme catalyses the cleavage of alginate chain by β-elimination and releases unsaturated uronic acid residues, i.e., mannuronic and guluronic acids. Different lyases can be more specific for different types of alginate blocks or uronic acids [29]. Alginate lyases have been used to evaluate alginate structure [30] as well as for the preparation of oligouronates [31,32]. The *P. alginovora* alginate lyase used in this study performs β-elimination on uronic acids residues from M–M, G–G and M–G diads of alginates and does not break down polyguluronate blocks [19]. However, enzymatic depolymerization was not complete and the quantity of alginate appeared underestimated. A mixture of oligo-alginates with degrees of polymerization (DPs) of 2, 3, 4, 5 and 6 with a majority of DP 3 and DP 4 was obtained. No monomers were produced. Also, enzymatic depolymerization was followed by a 2-h chemical hydrolysis with 1 *M* sulfuric acid at 100 °C. Only monomers of uronic acids were integrated. However, hydrolysis was not quantitative. The sensitivity of the method was improved by increasing the time of chemical hydrolysis step without evidence of uronic acids degradation, followed by chemical hydrolysis to obtain only DP 1 and DP 2 (not shown). Monomers and dimers were integrated and analyzed. Thus, the method can be used to detect lower concentrations of alginates in biological fluids.

This method was used to perform pharmacokinetic studies of sodium alginate and we showed that after an intravenous bolus injection of 20 mg/kg alginate the profile appears to be biphasic, suggesting a two-compartment model, with a half-life of approximately 2 h. These data are similar to those found by Skaugrud et al. [33] who described a comparable half-life of alginate following an intravenous bolus injection of 5 mg/kg alginate in mice.

Alginate is the most commonly used material for cell encapsulation and is well tolerated by various tissues. A wide spectrum of cells and tissues has been encapsulated and implanted, both in animals and humans, indicating the general applicability of this approach for both research and medical purposes. The feasability of transplanting cells within an immunoprotective membranes is currently being studied for the treatment of a wide variety of diseases, including anemia [34], dwarfism [35], hemophilia B [36], and both kidney [37] and liver failure [38], pituitary [39] and central nervous system [40] and diabetes mellitus [41]. Host reactions against therapeutic cells of foreign origin were neutralized by alginate encapsulation, thus preventing the ingress of high molecular mass substances such as immune cells, and secretory substances, such as cytokines, immunoglobulins, and complement factors. Therefore, microencapsulation technologies using alginate provides a safe and simple technique for implanting cells into various sites of the human body or for inoculating in extracorporeal bioartificial organs.

In conclusion, we have described a reliable and sensitive method for the detection of alginate in biological fluids, based on HPAEC–PAD of uronic acid residues following enzymatic depolymerization by alginate lyase. This method appears suitable in cell therapy, for monitoring possible release of alginate from entrapped cells following either their implantation within tissues or their inoculation in hybrid artificial organs, such as extracorporeal bioartificial liver.

Acknowledgements

M.D. and N.A. equally contributed to the study. The authors thank P. Hamon, S. Georges, A. Maubois, G. Chartier, and B. Saclier for technical support, and A. Guillouzo for critical reading of the manuscript. This work was supported by INSERM and by grants from Ministère de la Recherche (Direction de la Technologie) and Fondation de l'Avenir (Paris, France).

References

[1] A. Haug, B. Larsen, O. Smidsrød, Carbohydr. Res. 32 (1974) 217.
[2] S. Sugawara, T. Imai, M. Otagiri, Pharm. Res. 11 (1994) 272.
[3] I.R. Matthew, R.M. Browne, J.W. Frame, B.G. Millar, Br. J. Oral Maxillofac. Surg. 31 (1993) 165.
[4] S.E. Barnett, S.J. Varley, Ann. R. Coll. Surg. Engl. 69 (1987) 153.
[5] P. Vanstraelen, Burns 12 (1992) 145.
[6] J.F. Guo, G.W. Jourdian, D.K. MacCallum, Connect. Tissue Res. 19 (1989) 277.

[7] O. Smidsrod, G. Skjak-Braek, Trends Biotechnol. 8 (1990) 71.
[8] C.K. Colton, Cell Transplant. 4 (1995) 415.
[9] K.T. Paige, L.G. Cima, M.J. Yaremchuk, B.L. Schloo, J.P. Vacanti, C.A. Vacanti, Plast. Reconstr. Surg. 97 (1996) 168.
[10] K.I. Draget, G. Skjak-Braek, O. Smidsrod, Int. J. Biol. Macromol. 21 (1997) 47.
[11] T.M.S. Chang, Science 146 (1964) 524.
[12] A. Joly, J.F. Desjardins, B. Frémond, M. Desille, J.P. Campion, Y. Mallédant, Y. Lebreton, G. Semana, F. Edwards-Levy, M.C. Levy, B. Clément, Transplantation 63 (1997) 795.
[13] B. Frémond, C. Malandain, C. Guyomard, C. Chesné, A. Guillouzo, J.P. Campion, Cell Transplant. 2 (1993) 453.
[14] M. Desille, S. Mahler, P. Seguin, Y. Mallédant, B. Frémond, V. Sébille, A. Bouix, J.F. Desjardins, A. Joly, J. Desbois, Y. Lebreton, J.P. Campion, B. Clément, Crit. Care Med. 30 (2002) 658.
[15] Y. Hagihara, Y. Saitoh, H. Iwata, T. Taki, S. Hirano, N. Arita, T. Hayakawa, Cell Transplant. 6 (1997) 527.
[16] L.P. Le Tallec, C. Korwin-Zmijowska, M. Adolphe, Cell Biol. Toxicol. 13 (1997) 95.
[17] C. Yu, M. Cunningham, C. Rogers, I.D. Dinbergs, E.R. Edelman, J. Pharm. Sci. 87 (1998) 1300.
[18] F.A. Johnson, D.Q. Craig, A.D. Mercer, J. Pharm. Pharmacol. 49 (1997) 639.
[19] F. Chavagnat, C. Duez, M. Guinand, P. Potin, T. Barbeyron, B. Henrissat, J. Wallach, J.M. Ghuysen, Biochem. J. 319 (1996) 575.
[20] Y.C. Lee, J. Chromatogr. A 720 (1996) 137.
[21] E. Landberg, A. Lundblad, P. Pahlsson, J. Chromatogr. A 814 (1998) 97.
[22] T. Suzuki, S. Watanabe, J. Chromatogr. 541 (1991) 359.
[23] S. Honda, E. Akao, S. Suzuki, M. Okuda, K. Kakehi, J. Nakamura, Anal. Biochem. 180 (1989) 351.
[24] K.R. Anumula, Anal. Biochem. 220 (1994) 275.
[25] H. Kwon, J. Kim, Anal. Biochem. 215 (1993) 243.
[26] S. Honda, T. Ueno, K. Kakehi K, J. Chromatogr. 608 (1992) 289.
[27] A. Guttman, K.W. Ulfelder, J. Chromatogr. A 781 (1997) 547.
[28] T.R. Cataldi, C. Campa, G.E. De Benedetto, Fresenius J. Anal. Chem. 368 (2000) 739.
[29] K. Østgaard, S.H. Knutsen, N. Dyrset, I.M. Aasen, Enzym. Microbiol. Technol. 15 (1993) 756.
[30] K. Østgaard, Carbohydr. Polym. 19 (1992) 51.
[31] Y. Yonemoto, H. Tanaka, Y. Yamashita, Y. Kitabatake, Y. Ishida, A. Kimura, K. Murata, Ferment. Bioeng. 75 (1993) 68.
[32] M. Natsume, Y. Kamo, M. Hirayama, T. Adachi, Carbohydr. Res. 258 (1994) 187.
[33] O. Skaugrud, A. Hagen, B. Borgersen, M. Dornish, Biotechnol. Genet. Bioeng. Rev. 16 (1999) 23.
[34] J. Koo, T.M. Chang, Int. J. Artif. Organs 16 (1993) 557.
[35] P.L. Chang, N. Shen, A.J. Westcott, Hum. Gene Ther. 4 (1993) 433.
[36] H.W. Liu, F.A. Ofosu, P.L. Chang, Hum. Gene Ther. 4 (1993) 291.
[37] D.A. Cielinski, H.D. Hurnes, Biotechnol. Bioeng. 43 (1994) 678.
[38] T.M. Chang, Trans. ASAIO J. 38 (1992) 128.
[39] P. Aebischer, P.C. Russell, L. Christenson, G. Panol, J.M. Monchik, P.M. Galletti, ASAIO Trans. 32 (1986) 134.
[40] P. Aebischer, M. Godard, A.P. Signore, R.L. Timpson, Exp. Neurol. 126 (1994) 151.
[41] F. Lim, A.M. Sun, Science 210 (1980) 908.