Determination of cholesterol, collagen, chondroitin sulphate A and phospholipids using Fourier transform near infrared reflectance spectroscopy with a right-angled light exit 400 μm thin fibre optic strand

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Introduction

Detailed examination of human arteries, especially of coronary arteries, in respect to the narrowing of the lumen, the consistency and the special features of the arterial wall, can be performed well using the most recent catheter techniques. Until now, however, the chemical composition of atherosclerotic plaques could not be determined without surgical intervention. Atherosclerotic lesions are heterogeneous. A sudden rupture of the plaque, followed by a local obstructive thrombosis, is the most prominent cause of an acute coronary event. The risk of plaque disruption, indeed, depends more on its composition and vulnerability than on the degree of stenosis. In a practical sense, knowledge of the chemical composition of these plaques could influence therapeutic strategies significantly. To do this, questions need to be addressed as to the cholesterol and collagen (and also chondroitinsulphate A and phospholipids) content of the plaques, the consistency of the fibrotic caps on the atheromas, the extent of complications such as necrosis and calcification in the plaques and the intensity of inflammatory reactions. In particular, therapeutic regimens, such as LDL apheresis and intensive drug treatment, could be influenced by such specific knowledge.[1–4]

In a previous work[5] we were able to prove that cholesterol can be determined in human aortic samples by near infrared (NIR) spectropscopy using commercial fibre optic strands (diameter 4 mm and 1 mm). Based on these results the current studies were done with a right-angled light exit fibre optic strand (diameter 400 μm), which is intended to be integrated into a catheter.

The aim of this investigation was to develop a 400 μm thin fibre optic strand with a light exit right-angled to the direction of the fibre optic strand. We examined whether NIR reflection spectroscopy, used in this way, is an acceptable tool for quantitatve determination of the main compounds of aortic walls (cholesterol, collagen, chondroitinsulphate A and phospholipids). We investigated non-aqueous and aqueous model mixtures of the main compounds of aortic walls and human aortic samples.

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Table 1. Composition of the aqueous model mixtures.

Sample No.	Cholesterol % (w / w)	Collagen % (w / w)	Chondroitin sulphate A % (w / w)	Phospholipids (Lecithin : Sphingomyelin = 3 : 1) % (w / w)	Water % (w / w)
01	1.9	17.2	5.0	0.3	75.6
02	2.2	14.7	8.0	0.4	74.7
03	1.9	17.6	8.7	0.3	71.5
04	2.3	15.1	7.7	0.3	74.6
05	2.6	20.5	6.2	0.3	70.4
06	2.3	22.4	6.5	0.4	68.4
07	1.7	17.0	8.6	0.3	72,4
08	1.3	18.3	6.4	0.3	73.7
09	1.7	18.6	8.9	0.3	70.5
10	1.2	19.7	5.3	0.3	73.5
11	10.4	7.8	5.3	1.2	75.3
12	13.6	4.0	4.6	1.0	76.8
13	6.7	9.5	4.8	2.7	76.3
14	11.2	11.5	2.7	1.2	73.4
15	10.9	6.5	6.5	1.0	75.1
16	6.6	8.2	5,6	1.3	78.3
17	5.9	12.2	4.3	1.7	75.9
18	5.8	9.2	4.1	2.9	78.0
19	6.0	13.9	4.2	2.0	73.9
20	5.8	10.9	6.2	2.3	74.8
21	24.2	6.4	1.9	3.2	64.3
22	17.9	3.0	2.1	3.2	73.8
23	26.0	4.1	4.1	3.5	62.3
24	19.9	4.0	2.6	2.5	71.0
25	21.6	5.2	1.5	2.8	68.9
26	23.7	4.3	1.3	1.5	69.2
27	12.9	3.2	2.8	1.9	79.2
28	19.2	2.3	3.4	3.0	72.1
29	8.3	3.2	4.7	3.3	80.5
30	13.7	4.7	4.8	2.2	74.6

Table 2. Composition of the non-aqueous model mixtures.

Sample No.	Cholesterol % (w / w)	Collagen % (w / w)	Chondroitin sulphate A % (w / w)	Phospholipids (Lecithin : Sphingomyelin = 3 : 1) % (w / w)
01	7.9	70.7	20.2	1.2
02	8.5	59.0	31.1	1.4
03	8.9	50.2	39.6	1.3
04	8.9	60.2	29.8	1.1
05	8.7	70.0	20.4	0.9
06	6.8	72.4	19.7	1.1
07	6.0	62.9	29.5	1.6
08	3.6	54.0	41.8	0.6
09	5.4	65.0	28.6	1.0
10	4.3	75.5	19.1	1.1
11	40.2	34.8	20.3	4.7
12	43.0	39.3	14.5	3.2
13	21.7	37.7	31.9	8.7
14	40.0	46.3	9.5	4.2
15	41.8	29.6	25.0	3.6
16	25.5	47.8	21.5	5.2
17	20.0	59.7	14.6	5.7
18	17.8	39.5	33.8	8.9
19	19.0	61.4	13.2	6.4
20	19.4	52.2	20.6	7.8
21	60.8	26.3	4.8	8.1
22	60.5	21.6	7.2	10.7
23	61.9	19.9	9.8	8.4
24	60.6	23.8	8.0	7.6
25	61.9	25.7	4.3	8.1
26	38.0	52.0	4.6	5.4
27	42.6	41.7	9.4	6.3
28	36.1	6.8	48.3	8.8
29	26.1	10.1	53.5	10.3
30	34.5	11.8	48.3	5.4

Material and methods

All measurements were based on near infrared diffuse reflection spectroscopy. The NIR spectra were measured by an IFS 28 FT-spectrophotometer (Bruker Analytik GmbH, Karlsruhe, Germany). The optical components were as follows: wavelength 1000–2500 nm (10000–4000 cm^{-1}), CaF_2 beamsplitter, InGaAs detector, spectral resolution 16 cm^{-1}, scans 64 and a fibre optic strand (length = 1.5 m and diameter = 400 µm). The top of the fibre optic strand was sanded down to an angle of 45° and mirror-coated on the reverse side (Co. Sentronic GmbH, Dresden, Germany).

The quantitative evaluations were performed by the chemometric partial least squares method (PLS). The calculations were carried out with the OPUS software QUANT 2 (Bruker Analytik GmbH, Karlsruhe, Germany).

The following substances were used for the production of the model mixtures: cholesterol, collagen, chondroitinsulphate A, phosphatidylcholine, sphingomyelin (Sigma–Aldrich Chemie GmbH, Munich, Germany).

The model mixtures were prepared by weighing the single compounds using a precision scale and by homogenisation. The "phospholipids" part of the model mixtures was a mixture of phosphatidylcholine : sphingomyelin in the ratio of 3 : 1. As indicated in Tables 1 and 2, 30 mixtures, with different compositions, were prepared according to the composition of the arterial tissue.

Human aorta specimens were obtained at autopsy. The specimens were washed with Krebs physiological salt solution, dried briefly under nitrogen streams and stored at –20°C. Before use, the specimens were allowed to reach room temperature.

Results

With the right-angled light exit 400 µm thin fibre optic strand, developed in cooperation with Co. Sentronic we could record the typical NIR spectra of the pure compounds of the main components of aortic walls (results not shown). The internal validation of the determination of cholesterol, collagen, chondroitinsulphate A and phospholipid concentrations in different mixtures showed a close correlation between the concentration determined by weighing and the NIR results. The quantification of chondroitinsulphate A and phospholipids in aqueous mixtures is difficult (low concentration range).

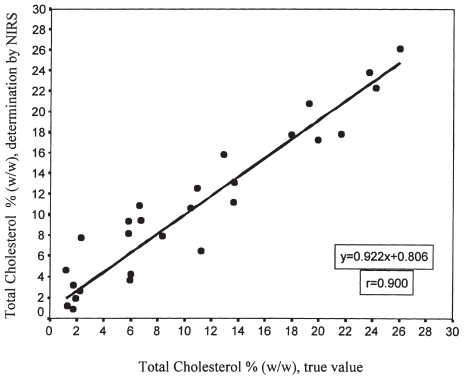

Figure 1. Determination of cholesterol. Correlation between the true value in the aqueous model mixtures and the determination by NIR.

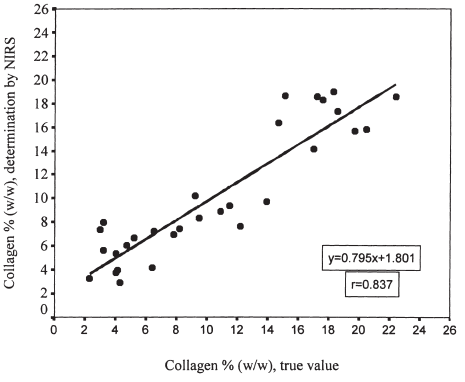

Figure 2. Determination of collagen. Correlation between the true value in the aqueous model mixtures and the determination by NIR.

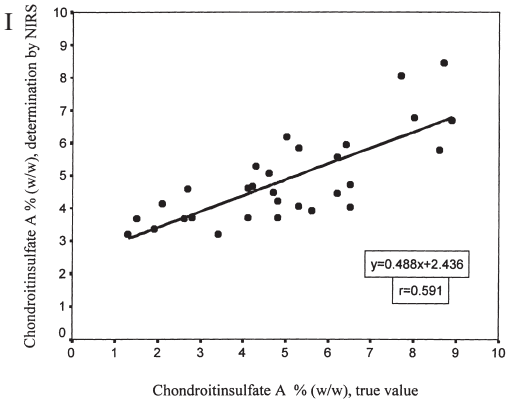

Figure 3. Determination of chondroitinsulphate A. Correlation between the true value in the aqueous model mixtures and the determination by NIR.

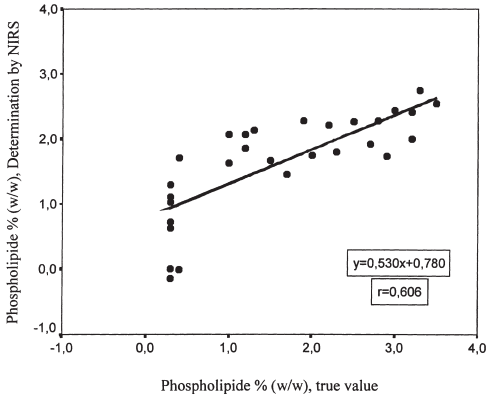

Figure 4. Determination of phospholipids. Correlation between the true value in the aqueous model mixtures and the determination by NIR.

n aqueous model mixtures specific absorption of the pure compounds were overlapped by strong OH absorptions.

For the main compound of the aortic wall the regression equations for the internal NIR reflectance spectroscopy validations were as follows:

Non-aqueous model mixtures:
Cholesterol r=0.962 , y=0.923 x + *2.15*
Collagen r=0.934 , y=0.895 x + 3.51
Chondroitin sulphate A r=0.908 , y=0.859 x + 2.95
Phospholipids r=0.883 , y=0.883 x + 0.58
Aqueous model mixtures (see also Figures 1–4)
Cholesterol r=0.900 , y=0.922 x + *0.81*
Collagen r=0.837 , y=0.795 x + *1.80*
Chondroitin sulphate A r=0.591 , y=0.488 x +2.44
Phospholipids r=0.606 , y=0.530 x + 0.78

The spectra of the human aortic samples showed a low signal-to-noise ratio and a poor reproducibility. It is not possible, at the moment, to determine cholesterol in human aortic samples with this fibre optic strand.

Discussion

NIR spectroscopy has been developed very intensively in the last decade, and the field of application has widened enormously. In biological science, NIR spectroscopy has become a well-accepted tool. Problems arising from the high water absorbency of tissues, from light scattering, from peak overlaps, and from peak shift have been overcome to a remarkable extent. The combination of a high standard of NIR spectroscopy with powerful computers appears to be an efficient tool for many applications *in vitro* as well as *in vivo*.6–11 Parallel to near infrared diffuse reflection spectroscopy, Raman spectroscopy has met increasing interest.[12–16]

Our results show that the recording of typical NIR spectra of the main compounds of the aortic wall (cholesterol, collagen, chondroitinsulphate A, phospholipids) is possible with our 400 µm thin fibre

optic strand with a right-angled light exit. The quantitative NIR determination of the main compounds, cholesterol and collagen, is possible in non-aqueous and aqueous model mixtures with a good correlation to reality, that means by weighing concentration. Compounds with a concentration less than 10%(w / w) (chondroitinsulphate A and phospholipids in aqueous model mixtures) can only be determined by NIR with a poor correlation to the true concentration. The main problems are the strong OH absorption and the poor signal-to-noise ratio. That is why, at the moment, we cannot determine cholesterol in human aortic specimens. But the quantitative determination of the main compounds of aortic wall in model mixtures is a precondition for the development of a coronary NIR catheter. Therefore, plenty of technical obstacles must be overcome.

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