This thesis deals with the development of a wearable glucose sensor for possible use in the treatment of diabetes mellitus.

In chapter one some general information on diabetes mellitus is presented as well as different strategies of medical treatment of the disease. Diabetes mellitus is a disease characterised by a relative or absolute deficiency of biologically active insulin. As a result carbohydrate and fat metabolism are deranged. Loss of control of the blood glucose level is of main concern, because of short-term and severe long-term complications. Therapy involves substitution of insulin to regain control of the blood glucose level. Normoglycaemia, though, can still not be achieved with multiple daily insulin injections. Several approaches to come to a more physiological insulin administration to improve diabetic treatment are discussed.

- Transplantation of the pancreas or isolated islets of Langerhans
- Open-loop insulin dosage systems, mainly concerning pre-programmable insulin pumps
- Closed-loop insulin dosage systems, in which a programmable insulin pump is controlled on the measurement of the actual body glucose concentration with the use of a glucose sensor. Final goal is to come to a complete artificial pancreas.

Problems related to the construction and realisation of a long-term reliable glucose sensor are discussed. An overview of all strategies presented so far to overcome these problems is given.

The design of the glucose sensor, presented in this thesis, is described in chapter two. The sensor consists of an oxygen electrode as detector and a dynamic enzyme microdialysis system as selector. Glucose is dialysed from the tissue using a hollow fiber membrane, which can be placed subcutaneously. Within the perfusion system the enzyme glucose oxidase catalyzes the reaction of glucose with oxygen. The reduced oxygen concentration of the dialysate is measured on-line with a Clark type oxygen electrode and can be related to the glucose concentration. In this design problems of biocompatibility, enzyme instability and oxygen limitation can be circumvented. In vitro response curves of the glucose sensor show a good correlation between the sensor output current and the glucose concentration. Linear effect of enzyme perfusion rate and hollow fiber membrane length could be demonstrated.

As the glucose sensor is based on the principle of microdialysis, a crucial part of the system is the hollow fiber membrane. The construction of these membranes is described in chapter three. The hollow fiber membrane is the only part of the system to be placed in the body. Therefore it has to be small and should be composed of inert materials. Membranes used in the construction of the hollow fiber should have pores, large enough to enable glucose diffusion into the microdialysis system, while enzyme diffusion into the body has to be prevented. Two types of tubular membrane were used:

- Polysulfone hollow fiber (MWCO 10,000 Dalton; internal diameter 0.5 mm, outer diameter 1.0 mm)
- Cellulose hollow fiber (MWCO 9,000 Dalton; internal diameter 150 μm, outer diameter 186 μm)
Several hollow fiber constructions are discussed. Improvements were made towards patient comfort, flow capacity and protection against enzyme leakage.

In chapter four different types of pumps are evaluated. The pump should be capable of delivering fixed flow rates, which have to be as constant as possible. All irregularities in the flow pattern have direct effect on the luminal glucose concentration of the system, thus disturbing the measurements. To achieve a wearable sensor the pump has to be miniaturizable and for continuous long-term use its energy consumption should be low. With the use of a roller pump or a piston pump it became possible to recirculate the glucose oxidase solution. Some adaptations, however, were necessary to maintain a reliable sensor signal. The oxygen consumed in the reaction had to be replenished. By introducing a gas-permeable tube after the electrode a constant basic oxygen concentration of the perfusion fluid could be established. The negative effect of hydrogen peroxide, formed in the enzymatic reaction, on the enzyme stability was eliminated by adding the enzyme catalase to the perfusion fluid to remove the hydrogen peroxide. In this way a closed loop microdialysis system was realised.

In chapter five the glucose sensor is tested in vivo in healthy volunteers. Twelve oral glucose tolerance tests were performed in which the subcutaneous glucose sensor signal was compared with the venous blood glucose concentration. Correlation between the sensor signal and the blood glucose concentration proved to be good. The delay observed between changes in glucose concentration in blood and subcutaneous tissue is not caused by the glucose sensor, but is a result of the temporary disturbance of the equilibrium between the intravascular and intercellular compartment with rapidly changing glucose concentrations. Calibrating the sensor signal on steady state blood glucose level resulted in lower calibration factors than those determined in vitro for the same hollow fiber. This suggested that subcutaneous tissue glucose levels are lower than venous blood glucose levels. In one experiment it was shown that the glucose sensor still measured reliably nine days after insertion of the hollow fiber.

In chapter six the glucose sensor is tested in diabetic patients. Asked to omit their morning insulin injection the patients arrived with high blood glucose levels. After placing the glucose sensor, insulin was administered intravenously resulting in a very fast decline of blood glucose concentration. With these experiments the maximum delay between the subcutaneous sensor signal and the venous blood glucose concentration could be determined, being 22 minutes. Despite this delay, correlation between the glucose sensor signal and the venous blood glucose concentration proved to be good. In some exceptional cases, though, flow irregularities and poor sensor sensitivity disturbed the measurements.

Chapter seven deals with the aspect of calibration of the glucose sensor. In vivo experiments were performed in twelve healthy volunteers and twelve diabetic patients. Blood glucose concentration was clamped at two different levels allowing a two point calibration of the sensor signal. Before and after each test the glucose sensor was calibrated in vitro. A good correlation was observed between the sensitivity of the sensor in vivo and in vitro, confirming the reliability of the glucose sensor microdialysis system. This is of particular interest, since glucose sensor calibration is a much discussed problem. No other research group so far could achieve any correlation between sensitivity in vitro and in vivo. This observed correlation in 24 humans also enabled a calculation of the absolute
glucose concentration in the subcutaneous tissue, being 43% of the blood glucose concentration. Furthermore, the low interindividual variation of the quotient vitro/vivo indicates that the subcutaneous abdominal fatty tissue is a good measuring site for glucose.

To validate the measurement of the actual subcutaneous glucose concentration with the glucose sensor, a different technique was developed to measure directly the subcutaneous glucose concentration, described in chapter eight. Sampling of subcutaneous intercellular fluid is established using a filtration technique in which a vacutainer is connected to subcutaneously inserted hollow fiber membranes.

Experiments were performed in 13 healthy volunteers. Apart from the subcutaneous filtration samples, glucose was followed subcutaneously with the glucose sensor and blood samples were taken as a reference. Initially the filtrate values were equal to blood glucose concentration, but after a six hour decline period, the value remained constant at 46% of the simultaneously determined blood glucose values. The glucose sensor, calibrated in vitro, measured 44% of the mean venous blood glucose concentration. The initially high glucose levels of the filtrate are explained to be caused by the disruption of local blood vessels and cells with the insertion of the membranes. Due to the suction applied on the membranes, wound healing is only completed after several hours after which the normal situation is restored and true interstitial glucose concentrations are measured. The close agreement between the two independent methods supports the statement that real glucose concentration in subcutaneous fatty tissue is about half of the blood glucose value. An anatomical model was developed, which made it possible to explain most of the conflicting results and theories presented so far.

More information on the actual subcutaneous glucose concentration is gathered in chapter nine. A new method was developed to validate the anatomical model. Saline or glucose solutions in saline were brought in an ultrafiltration hollow fiber membrane, inserted into subcutaneous tissue. The fluid was allowed to equilibrate with its surrounding after which the sample was collected. Again, subcutaneous glucose concentration proved to be lower than the simultaneously measured blood glucose level. Several other in vivo experiments on humans and dogs made it possible to draw a final conclusion on the actual situation in the subcutaneous tissue. These results are of great importance, since knowledge on the actual amount of glucose present at the measuring site of the glucose sensor is a prerequisite for the successful introduction of such a device in diabetic treatment.