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13. ABSTRACT (Maximum 200 words)

We describe in this paper an experiment involving an electronically conductive polymer that, to our knowledge, has not been described previously. A free-standing conductive polymer (polypyrrole)-based membrane separates a solution of an electron donor from a solution of an electron acceptor. Because the conductive polymer is both electronically and anionically conductive, the membrane can transport electrons from the donor solution to the acceptor solution, and anions in the opposite direction, such that a sustainable electron-transfer reaction is driven across the conductive polymer membrane. We demonstrate such transmembrane electron/ion-transfer processes using both an inorganic and a biochemical electron donor/acceptor system. The biochemical case is of particular interest because we show that the reduced form of the enzyme glucose oxidase can give its electrons directly to the polypyrrole-membrane surface. Direct electron transfer is usually not possible at inorganic metals.

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INORGANIC AND BIOLOGICAL ELECTRON TRANSFER ACROSS AN ELECTRONICALLY CONDUCTIVE COMPOSITE POLYMER MEMBRANE

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ABSTRACT

We describe in this paper an experiment involving an electronically conductive polymer that, to our knowledge, has not been described previously. A free-standing conductive polymer (polypyrrole)-based membrane separates a solution of an electron donor from a solution of an electron acceptor. Because the conductive polymer is both electronically and anionically conductive, the membrane can transport electrons from the donor solution to the acceptor solution, and anions in the opposite direction, such that a sustainable electron-transfer reaction is driven across the conductive polymer membrane. We demonstrate such transmembrane electron/ion-transfer processes using both an inorganic and a biochemical electron donor/acceptor system. The biochemical case is of particular interest because we show that the reduced form of the enzyme glucose oxidase can give its electrons directly to the polypyrrole-membrane surface. Direct electron transfer is usually not possible at inorganic metals.

Electronically conductive polymers constitute an interesting class of synthetic metals (1). We describe in this paper an experiment involving an electronically conductive polymer membrane that, to our knowledge, has not been described previously. In this experiment, a free-standing conductive polymer-based membrane separates a solution of an electron donor from a solution of an electron acceptor (Figure 1). We show that because the conductive polymer is both electronically and anionically conductive, the membrane can transport electrons from the donor solution to the acceptor solution, and anions in the opposite direction, such that a sustainable electron-transfer reaction is driven across the conductive polymer membrane. We demonstrate such transmembrane concerted electron/ion-transfer processes using both an inorganic and a biochemical electron donor/acceptor system. The biochemical case is of particular interest because we show that the reduced form of the enzyme glucose oxidase can give its electrons directly to the conductive polymer surface. Direct electron transfer is usually not possible at conventional inorganic metals.

Polypyrrole (2) was used as the electronically conductive polymer; only the *doped* form of the polymer was used. Therefore, electron and ion transfer is *not* associated with oxidation of the undoped polymer as would be the case in an electrochemical experiment involving a polypyrrole film-coated electrode (2). In the electrochemical case, electrons and ions will flow across the polypyrrole film only until the polypyrrole is fully oxidized. In the experiment described here, electrons and ions will flow continuously across the membrane as long as unreacted donor and acceptor are available on the opposite sides of the membrane. This process is also mechanistically distinct from the case in which a doped polypyrrole film-coated electrode is used to drive a redox reaction at the film/solution interface (3) because, in

that case, only electrons are transported across the film. In a sense, the polypyrrole-based membranes in the experiments described here are analogous to the lipid membranes in biological electron transfer processes in that both electrons and ions are shuttled, in a concerted fashion, across the membrane. Of course, the polypyrrole-based membranes used here are chemically quite different from the biological membranes.

The membranes used for these investigations were obtained by synthesizing polypyrrole within the pores of a microporous polycarbonate host membrane; we have described this synthesis in detail elsewhere (4,5). The host membrane employed (4,5) had cylindrical, 0.4 μm-diameter pores. A composite with polypyrrole "wires" running through these pores is obtained (Figure 1). This composite membrane approach was taken because polypyrrole, and other conductive polymers, typically have poor mechanical properties. In contrast, the composite membranes that contain polypyrrole as the active component are durable and mechanically robust.

The donor solution for the inorganic electron-transfer system was 1M KI. The acceptor solution for the inorganic system was either 1M $FeCl_3$ or 1 M $Fe_2(SO_4)_3$. Solutions were stirred during the transport experiment. The donor solution for the biological electron transfer system contained 0.2 mg/mL of type VII glucose oxidase (Sigma), and was 20 mM in glucose. The acceptor solution was 48 μm in 2,6-dichlorophenol-indophenol (DPIP), a commonly-used electron acceptor for biological electron-transfer reactions. Both solutions were thoroughly degassed and were also 25 mM in KCl and buffered at pH = 7.0.

The transmembrane redox reaction for the inorganic system can be written as;

$$3\,I^- + 2\,Fe^{3+} \text{-----} > I_3^- + 2\,Fe^{2+} \qquad (1)$$

the appearance of the characteristic absorption bands for I_3^- was used to monitor the progress of the reaction in the donor half cell (Figure 2A). The complexing agent 2,3,4-tri(2-pyridyl)-1,3,5-triazine (TPTZ) was added to aliquots from the acceptor half cell; the Fe^{2+}-TPTZ complex shows a characteristic absorbance at 590 nm (Figure 2B). The transmembrane redox reaction in the biological system can be written as

$$FADH_2 + DPIP \text{ -----> } FAD + DPIPH_2 \qquad (2)$$

where $FADH_2$ is the reduced flavin cofactor for the enzyme and $DPIPH_2$ is the reduced form of the acceptor. As indicated above, $FADH_2$ was generated in situ by adding excess glucose to the donor half-cell. This transmembrane reaction was monitored via the disappearance of the DPIP absorbance at 602 nm (Figure 3).

It is conceivable that the redox reactions shown in Equations 1 and 2 occurred in these experiments via transport of the reactants across the membrane. However, after termination of the experiment the donor species could not be detected in the acceptor solution and vice-versa. These analyses show that, on the time scale of these experiments, the extent of transport of the reactants across the membrane is negligibly small. Hence, the results obtained here cannot be explained by simple transport of the reactants followed by homogeneous electron transfer in the donor or acceptor solution.

Figure 2 shows typical absorption data for both of the half-cells for the inorganic system. As would be expected, the absorbance increases for both I_3^- and the Fe^{2+}-TPTZ complex during the course of the experiment. The concentrations of I_3^- and Fe^{2+} produced were determined from such spectra. In perfect agreement with Equation 1, the stoichiometric ratio of Fe^{2+} to I_3^- produced was 2.0 ± 0.1. These data unambiguously prove

that the electron transfer reactions shown in Equation 1 is being driven in a heterogeneous fashion across the polypyrrole-based membrane. Again, to our knowledge, this type of experiment has not been described previously.

It is known that electronic conduction is faster than ionic conduction in polypyrrole (6). This suggests that anion-transport in the membrane might be the process that limits the rate of the transmembrane redox reaction. To explore the effect of anion transport on the rate of this transmembrane reaction, we compared the rate of generation of I_3^- in the donor half-cell when ferric chloride and ferric sulfate were used in the acceptor half-cell. If, as expected (6), anion transport is rate-limiting, the reaction rate should be lower when the larger and more highly-charged sulfate ion is used. Figure 3 shows that this is, indeed, the case. It is important to point out that while anion transport is expected to dominate over cation transport (because doped polypyrrole is a polycation), we cannot discount the possibility of a contribution by cation transport from the donor half cell to the acceptor half cell. The strong dependence of reaction rate on anion (Figure 3) indicates, however, that if cation transport does play a role, it is a minor role.

We now turn our attention to the more interesting case of biological electron-transfer. Figure 4 shows absorption spectra in the acceptor (DPIP) half-cell for the transmembrane redox reaction described by Equation 2. As would be expected, the DPIP absorbance decreases during the course of the experiment. That this decrease is caused by electron transfer to DPIP is proven by the fact that the DPIP absorbance can be regenerated by simply bubbling air through the acceptor solution. This, again, shows that the loss of DPIP absorbance is not simply due to physical loss of this molecule from the acceptor half-cell.

To explore the role of the biocatalyst (glucose oxidase) in this electron transfer process, we conducted analogous experiments with just glucose in the donor solution. While transmembrane DPIP reduction did occur, the rate of reduction was slower than when the biocatalyst was added to the donor solution. These results prove that the reduced form of glucose oxidase can give its electrons directly to the conductive polymer surface. This is typically not possible at an inorganic metal surface (7). It is interesting to note that Nolte et al. have recently presented results of an electrochemical experiment which also suggests that direct electron transfer between glucose oxidase and polypyrrole is possible (8).

The fact that the transmembrane electron-transfer reaction occurred (albeit at a lower rate) in the absence of glucose oxidase was a surprising result. We have since shown that doped polypyrrole can catalyze the oxidation of glucose. We will report on these interesting results in a following paper.

A number of interesting question arises from this work. These include - 1) What is unique about this conductive polymer that allows it to participate in direct redox reactions with the enzyme? The experiment described here is uniquely suited for exploring this important fundamental question. 2) Is this unique to polypyrrole and glucose oxidase or can other redox enzymes react at other conductive polymer surfaces? 3) Can an enzymatic donor and an enzymatic acceptor be linked via a membrane of this type? If so, these membranes might find uses in novel bioreactor systems. 4) If a donor with a very negative E° is used will the polymer become undoped an thus shut off the electron-transfer pathway? We are currently exploring these questions.

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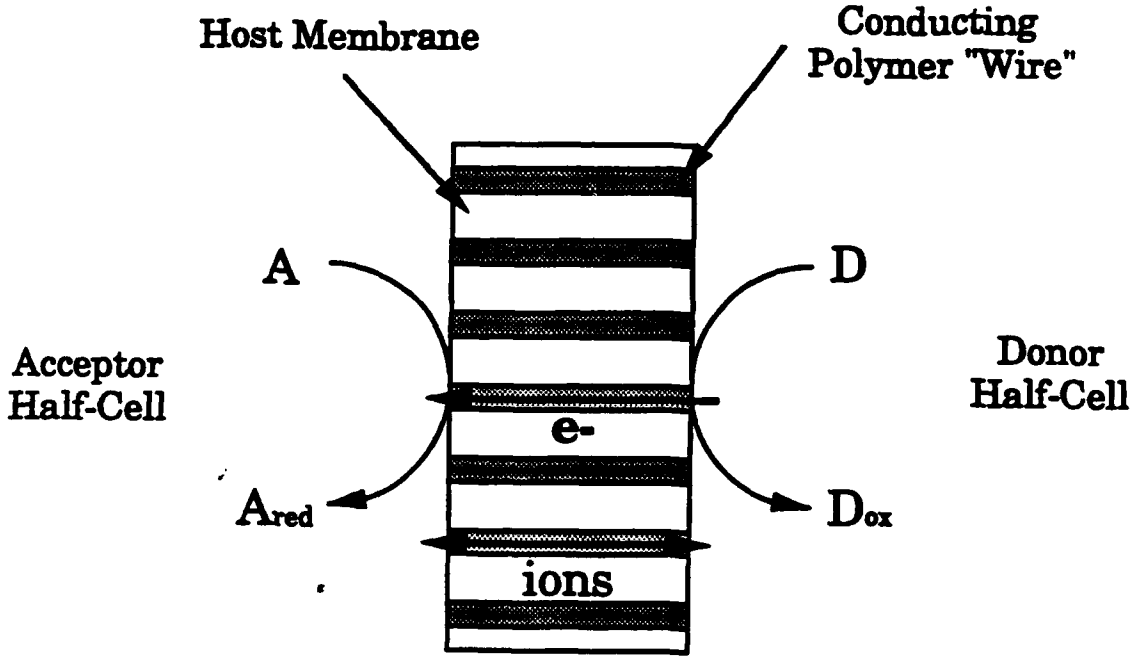
Figure captions.

Figure 1. Schematic representation of the cross-section of the electronically-conductive composite polymer membrane and of a hypothetical transmembrane electron-ion transport process. A/A_{red} = electron acceptor redox couple; D/D_{ox} = electron donor redox couple.

Figure 2. Absorption spectra of the products of the transmembrane redox reaction given by Equation 1 at various times after initiation of the experiment. (A) I_3^- absorption from the electron donor half-cell and (B) Fe^{2+}-TPTZ complex from the electron acceptor half-cell.

Figure 3. Comparison of the rate of the transmembrane redox reaction (Equation 1, as indicated by the rate of I_3^- formation in the donor half-cell) when Cl^- (upper) and SO_4^{2-}(lower) is the anion in the acceptor half-cell.

Figure 4. DPIP absorbance in the acceptor half-cell at various times during the transmembrane redox reaction experiment described by Equation 2.


Host Membrane
Conducting
Polymer "Wire"
A
D
Acceptor
Half-Cell
Donor
Half-Cell
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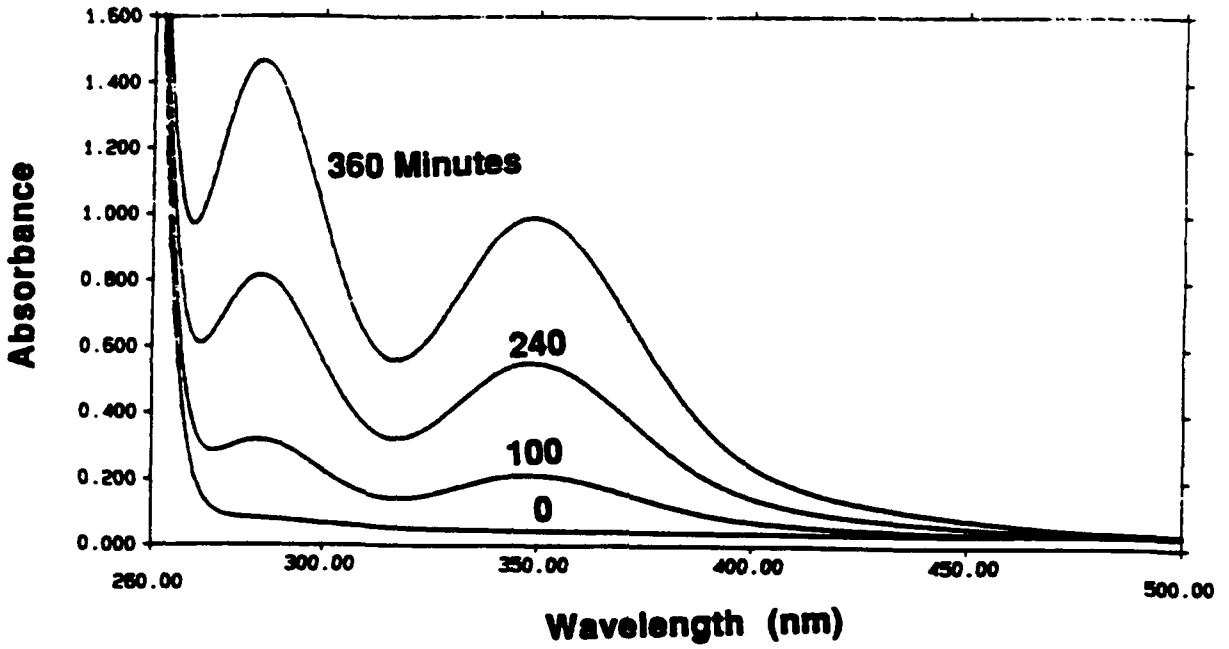
Dox
ions

Fig. 1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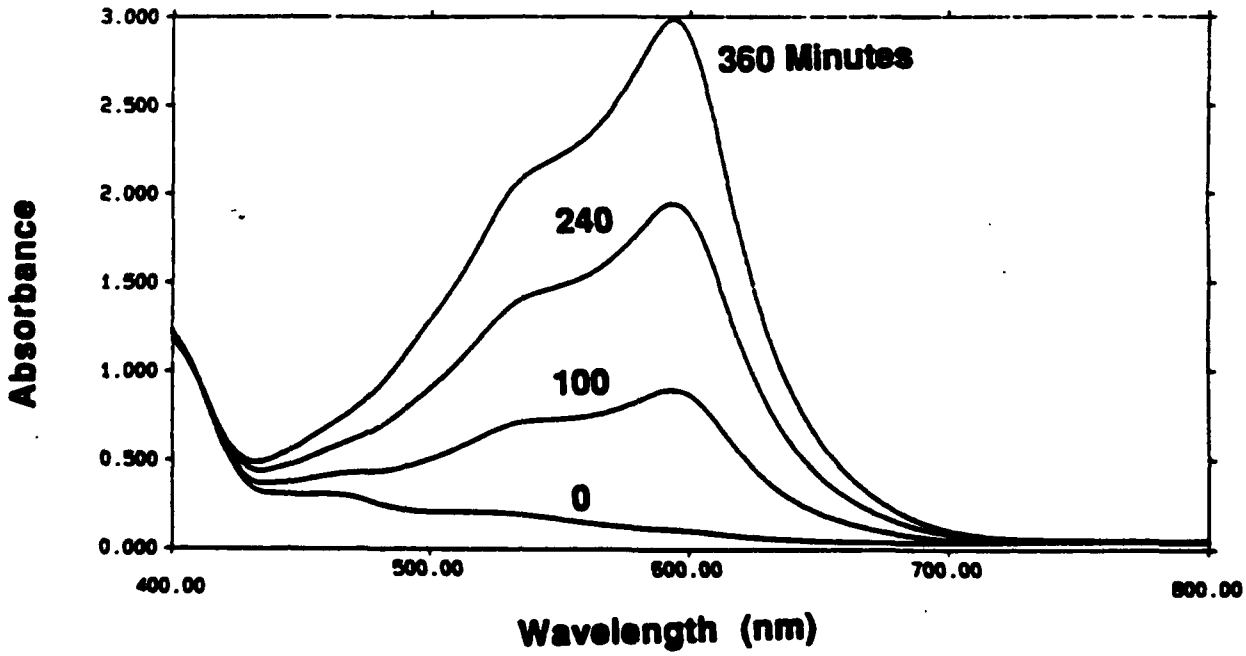
Wavelength (nm)

A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Wavelength (nm)

B

Fig. 2

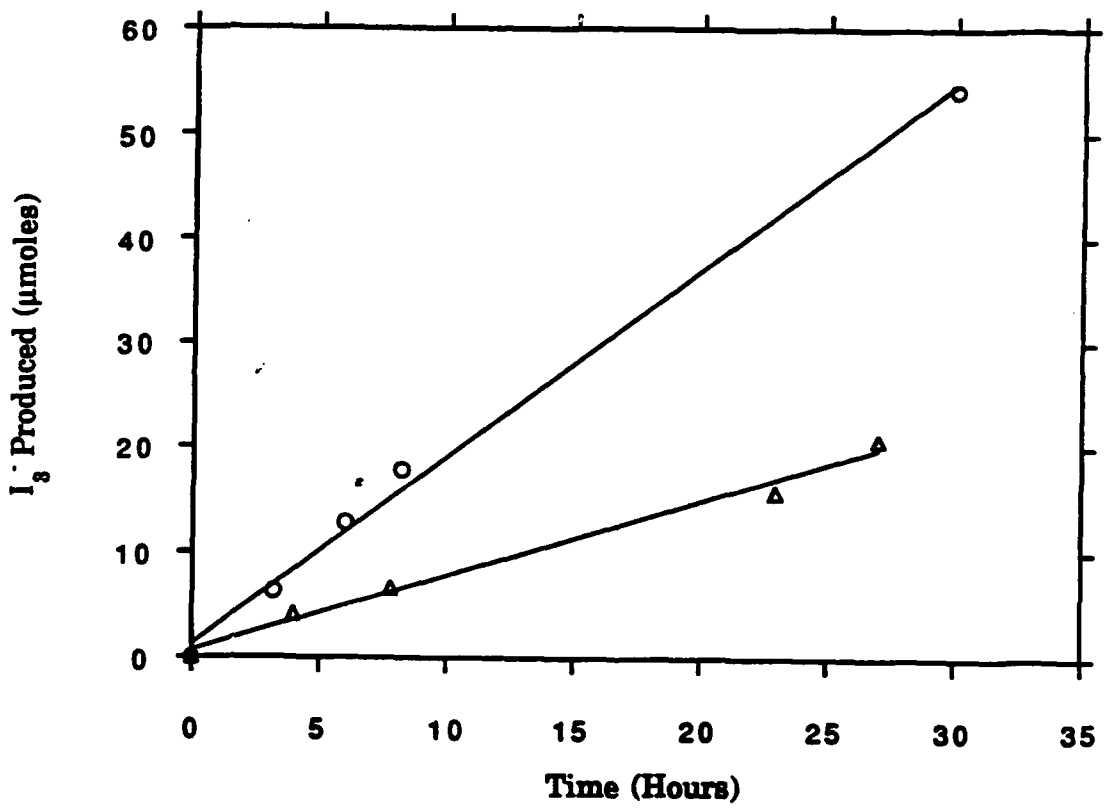
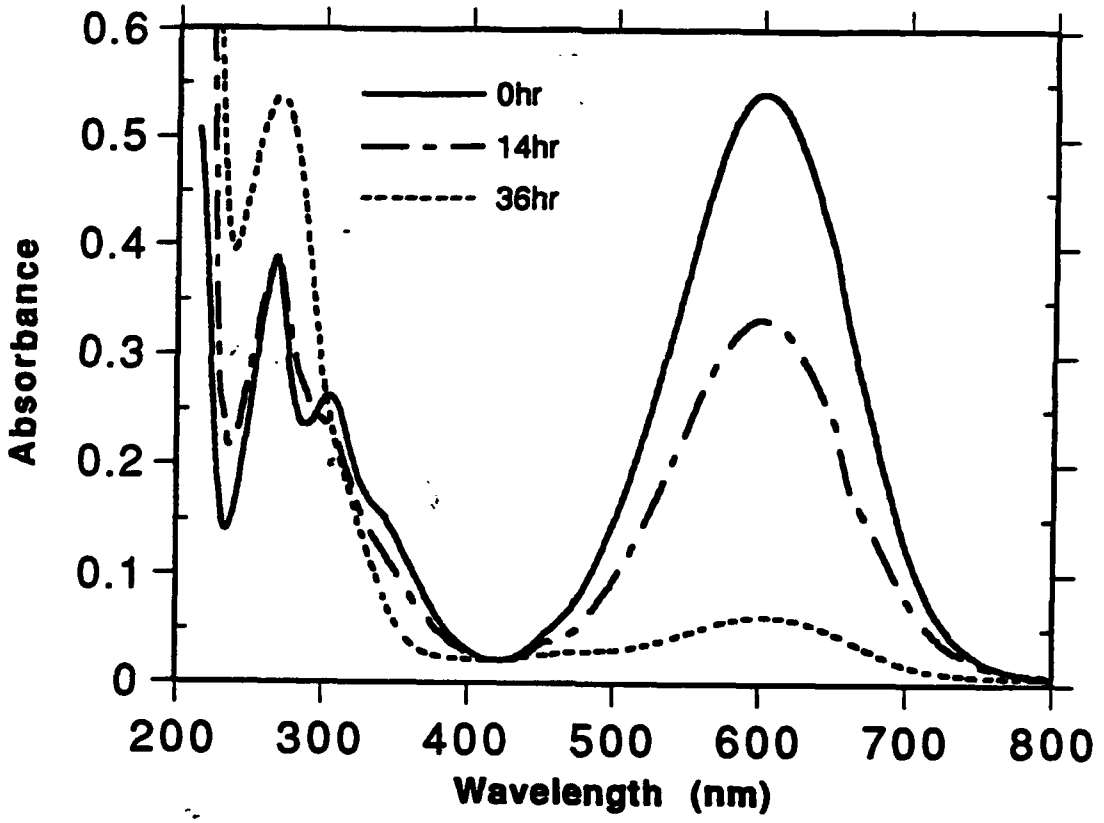

60
50
40
30
20
10
0
I_3^- Produced (µmoles)
0
5
10
15
20
25
30
35
Time (Hours)

Fig. 3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0.3
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0
Absorbance
0hr
14hr
36hr
200
300
400
500
600
700
800
Wavelength (nm)

Fig. 4.