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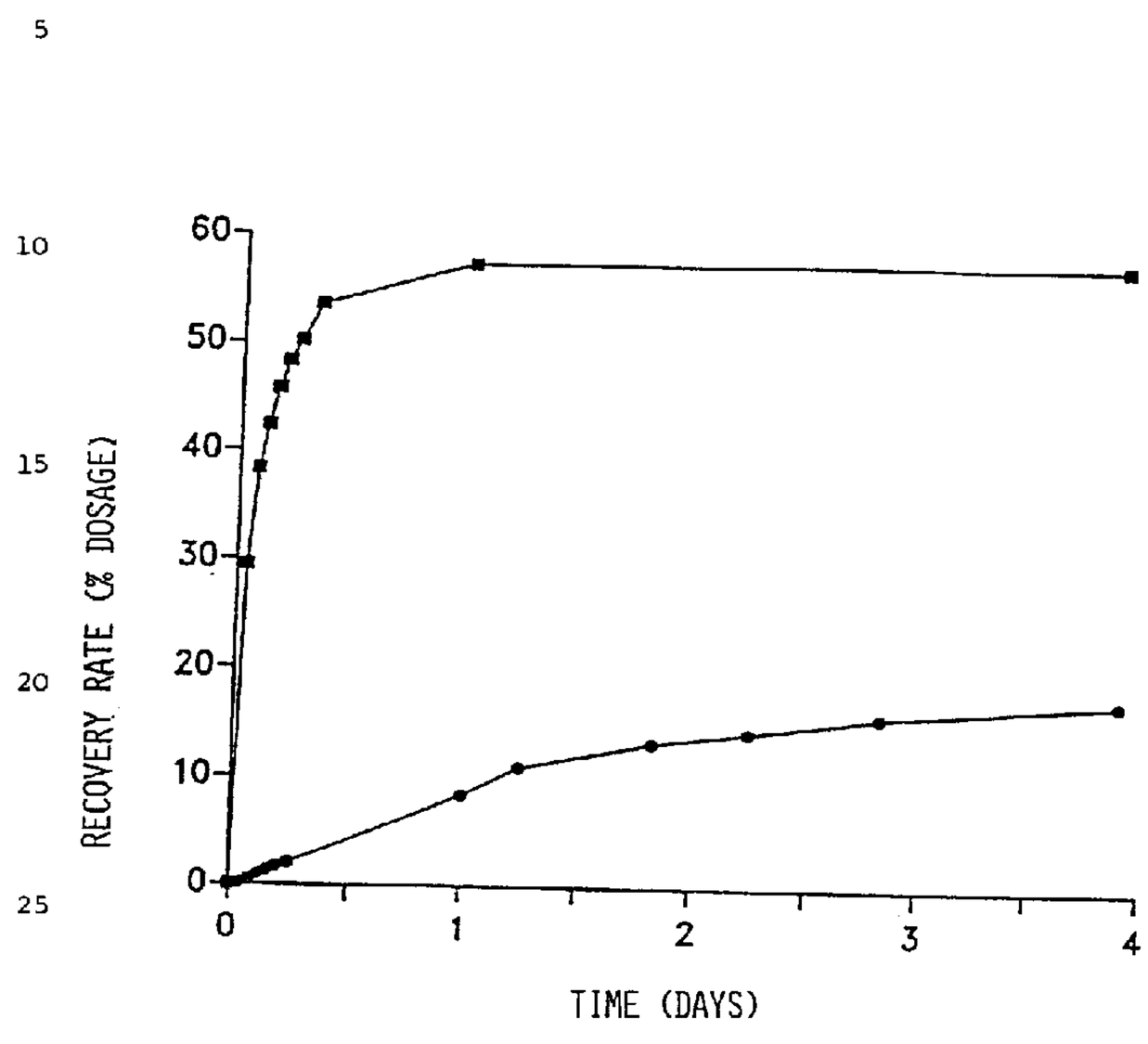
(54) **UTILISATIONS DE MICELLES MELANGEES**
(54) **USE OF MIXED MICELLES**



(57) Mixed micelles can be used as carriers for the parenteral administration of pharmaceutically active substances. Mixed micelles carry the active substances directly into the lymphatic system. As an example, it has been found that the use of the mixed micelle systems as carriers for administering immunomodulators and cytostatics, increases the bioavailability of these active compounds.

Abstract

Mixed micelles can be used as carriers for the parenteral administration of pharmaceutically active substances. Mixed micelles carry the active substances directly into the lymphatic system. As an example, it has been found that the use of the mixed micelle systems as carriers for administering immunomodulators and cytostatics, increases the bioavailability of these active compounds.

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The present invention is concerned with the use of mixed micelles as carriers for the parenteral administration of pharmaceutically active substances as well as mixed micelle solutions which contain certain pharmaceutically active substances.

It is known to use mixed micelles as a vehicle for pharmaceutically active substances. DE-PS 27 30 570 describes mixed micelle solutions for the parenteral administration of fat-soluble active substances, i.e. active substances which are not soluble in water or which are difficultly soluble in water, especially benzodiazepines and vitamin K. EP-A2-133 258 describes mixed micelle solutions for the parenteral administration of vitamin E. Furthermore, mixed micelles have been proposed for the production of aqueous protein solutions (EP-A1-252004). In the use of mixed micelles for the solubilization of non-steroidal antiinflammatories in accordance with EP-A1-280 887 the problem lay in improving the tolerance of the active substances in the case of injections.

In the case of parenteral, especially interstitial, e.g. intramuscular, subcutaneous or intradermal, administration of pharmaceutically active substances, those having a molecular weight <1000 can be taken up lymphatically, while active substances whose molecular weight amounts to about 16000 or above are taken up to a substantial extent from the lymphatic system.

It can, however, be desirable also to introduce

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low-molecular active substances, which are normally not transported to a substantial extent by the lymphatic system, into this and thus to transport them to the destination. This is especially true in the case of active substances which influence the immune system, such as immunosupressants and immunostimulants. It has surprisingly been found that mixed micelles are transformed in the case of interstitial applications into liposomes which are taken up and transported by the lymphatic system. In the case of certain hypotheses which have been put forward, active substances contained in mixed micelles are taken up by the liposomes formed in the organism. Whether in each case such hypotheses are proved can be ascertained experimentally by determining the distribution coefficient of the active substance in octanol/water. Active substances whose distribution coefficient in the system octanol/water is greater than about 10^4, preferably greater than about 10^6, fulfil the hypothesis, i.e. are taken up by the liposomes which are formed.

Fat-soluble vitamins, e.g. vitamins A, E and K, have a distribution coefficient of $>10^6$ in octanol/water. As mentioned above, mixed micelle solutions of such vitamins have become known. However, it has not become known that these vitamins, after parenteral, especially interstitial, administration as mixed micelle solutions, pass into the lymphatic vessels in the form of liposomes.

The object of the invention is accordingly the use of mixed micelles as carriers for the parenteral, especially interstitial, therapeutic administration of low-molecular active substances having a distribution coefficient in octanol/ water of greater than about 10^4, with fat-soluble vitamins being excluded.

As low-molecular active substances there are to be understood in the present connection active substances having a molecular weight below 70000. The low-molecular active substances preferably have a molecular weight below 5000.

Examples of active substances in the case of whose parenteral, especially interstitial, therapeutic administration the use in accordance with the invention of mixed micelles as carriers comes into consideration are immunomodulators, e.g. immunosuppressants such as (all-E)--3,7-dimethyl-9-(2-trifluoromethyl)-6-(nonyloxy)phenyl--2,4,6,8-nonatetraenoic acid and related compounds which are described in EP-A1-169 571; cyclosporin or FK 506 (Immunology Today 10, 6-9 (1989)); as well as lipophilic derivatives thereof, e.g. C_{1-20}-alkanoyloxy derivatives; immunostimulants such as N-acetylmuramyl-L-alanyl-D--isoglutamyl-L-alanyl-2-(1',2'-dipalmitoyl)phosphatidyl--ethanolamine (Cancer Immunol. Immunother. (1986) 22: 191-196); peptide antigens and oligosaccharide antigens or lipophilic derivatives thereof, e.g. peptide-fatty acid conjugates (EP-A2-290 246); contrast agents for lymphatic scintillography; cytostatics, e.g. cis-Pt complexes such as cis-bis-N-decylaminodiacetato-1,2-diaminocyclohexane--platinum; or anthra- cyclines such as doxorubicin or methotrexate or lipophilic derivatives thereof such as the fatty acid esters mentioned above, or lipophilic derivatives of 5-fluoro-2'-deoxyuridine, e.g. palmitoate (Microencapsulation 1988, Vol. 5, No. 1, p. 1-11) and phosphate (Japan. Kokai 1091 195); lipophilic derivatives of 1-ß-D-arabinofuranosyl-cytosine (Ara-C) such as oleate and palmitate (Int. J. Cancer 37, 149-154 [1986]) and cholesteryloxycarbonylglycyl-Ara-C (Chem. Pharm. Bull Vol. 36 (1988) 4060 et seq.); phosphatidylinositol and lysophospholipids (DE-OS 3008082); and anti-infectives such as amphotericin B, nystatin, gentamycin, chloroquin,

penicillins such as ampicillin; and tetracyclines or lipophilic derivatives thereof such as the fatty acid esters mentioned above.

The previously named active substances can be incorporated in mixed micelles using technologies known per se, for example as described in DE-PS 27 30 570. In comparison to liposomal solutions, mixed micelle solutions are simpler to manufacture and have an improved stability. The mixed micelle solutions manufacturable in accordance with the invention therefore combine the advantage of the stability of mixed micelle solutions with the improved efficacy of lymphatically- and/or liposomally-applied active substance.

In the scope of the present invention there are to be understood under mixed micelles or mixed micelle solutions aqueous, homogeneous solutions of cholanic acid derivatives, especially alkali salts thereof and lipids. Examples of cholanic acid salts are Na cholate, glycocholate, taurocholate, deoxycholate, glyco- and taurodeoxycholate, chenodeoxycholate; and glyco- and taurochenodeoxycholate. Examples of lipids are natural, semi--synthetic and wholly-synthetic lipids, especially phosphatidylcholines, glycerol ether phosphatides, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin, plasmalogens, cardiolipin, sulphatides and monoglycerides. The mixed micelles can contain as additional components cholesterol (up to about 30 mol% based on the amount of lipid) and lipids having negatively- or positively-loaded groups such as phosphatidic acids or stearylamine (up to about 10 mol% based on the amount of lipid).

The mixed micelle solutions can be manufactured by simply mixing together the individual ingredients. It is,

however, advantageous to dissolve the lipid ingredient, the micelle former and the active substance(s), which is/are difficultly soluble in water or insoluble in water, in an organic solvent, then to evaporate the organic solvent and thereupon to add the water, the isotonizing additives and, if desired, the additional ingredients, whereby as a rule the isotonizing additives and for the most part also the optional additional ingredients are mixed with the water prior to the addition to the mentioned evaporation residue. As organic solvents there come into consideration those in which the components to be solubilized are sufficiently soluble, such as e.g. lower alkanols, especially ethanol.

An especially preferred procedure comprises stirring intensively approximately one molar part of micelle former, approximately one molar part of lipid ingredient and approximately 0.3 to one molar part of water, optionally in the presence of up to 2% of an organic solvent such as ethanol, as well as the active substance(s) which is/are difficultly soluble in water or insoluble in water until the mixture appears homogeneous, whereupon the water, the isotonizing additives and, if desired, the additional ingredients are added until the desired dilution or concentration has been achieved. It is, how- ever, also possible to carry out the above procedure with- out the active substance and only at the end to solubilize the active substance(s) in the micelle solution.

The ratio between the lipid ingredient and the micelle former lies in the order of 0.1:1 to 2:1. Mixture ratios of 0.1:1 to 0.8:1 and 1.5:1 to 2:1 are preferred. The mixture ratio 0.8:1 to 1.5:1 is quite especially preferred.

The amount of lipoid ingredient plus micelle former in

the injection solution can vary over wide limits and can amount to e.g. 2-200 mg/ml of injection solution.

The amount of the pharmacon in the mixed micelle solution can also vary over wide limits and can amount to e.g. 0.1-100 mg/ml of injection solution.

Antioxidants such as tocopherols, ascorbic palmitate, sodium ascrobate, sodium hydrogen sulphite, sodium pyrosulphite or sodium sulphite can be added to prevent oxidation reactions of the active substance and of the carrier materials.

Additional substances of pH-adjustment, e.g. phosphate, citrate or Tris buffer; for isotonization, e.g. sodium chloride, mannitol, sorbitol or glucose, and for preservation, e.g. methyl and propyl p-hydroxybenzoate, benzyl alcohol or phenol, can also be added.

Where desired, the mixed micelle solutions can be converted into dry preparations with the aid of conventional lyophilization procedures.

The following Examples are intended to illustrate the invention further.

Example 1

Manufacture of mixed micelle solutions:

Active substance (all-E)-3,7-dimethyl-9-(2-trifluoromethyl)-6-(nonyloxy)phenyl-2,4,6,8-nonatetraenoic acid (A)

30.8 mg of soya lecithin (Epikuron 200, Lucas Meyer, Hamburg, Germany), 21.40 mg of sodium glycocholate and 0.4 mg of (all-E)-3,7-dimethyl-9-(2-trifluoromethyl)-6-

-(nonyloxy)phenyl-2,4,6,8-nonatetraenoic acid are dissolved in 5 ml of chloroform/methanol (1:1, vol/vol%) in a round flask. The film which results after evaporation of the organic solvent (rotary evaporator, 40°C) is dispersed in 1 ml of 3.8% mannitol solution (wt/vol%). The micelles obtained are adjusted to pH 6.0 ± 0.1 with 1N HCl, sterilized, filled into vials and lyophilized. The mixed micelles are manufactured with the exclusion of light and in an inert gas atmosphere, e.g. under nitrogen, in order to prevent isomerization reactions of the active substance as well as oxidation reactions of the active substance and of the carrier materials, especially of the phospholipids.

Example 2

Active substance 3',5'-di-O-palmitoyl-5-fluoro-2'--deoxuridine (B)

30.8 g of soya lecithin (Epikuron 200, Lucas Meyer, Hamburg, Germany), 21.67 mg of sodium glycocholate and 1.0 mg of 3',5'-di-O-palmitoyl-5-fluoro-2'-deoxyuridine are dissolved in 5 ml of chloroform/methanol (1:1, vol/vol%) in a round flask. The film which results after evaporation of the organic solvent (rotary evaporator, 40°C) is dispersed in 1 ml of 3.8% mannitol solution. The micelles obtained are adjusted to pH 6.0 ± 0.1 with 1N HCl, sterilized and filled into ampoules. The manufacture of the micelles is carried out in an inert gas atmosphere, e.g. under nitrogen, in order to prevent oxidation reactions of the active substance and of the carrier materials, especially of the phospholipids.

The mixed micelle solutions were administered intradermally to the right hind leg of experimental animals (sheep) by means of a device described in EP-A-272530.

Blood was removed through a jugular vein catheter and lymphatic fluid was removed through a cannula in the branching lymph vessel at the right popliteal lymph node (Pharm. Res. Vol. 5, 472-476 (1988)).

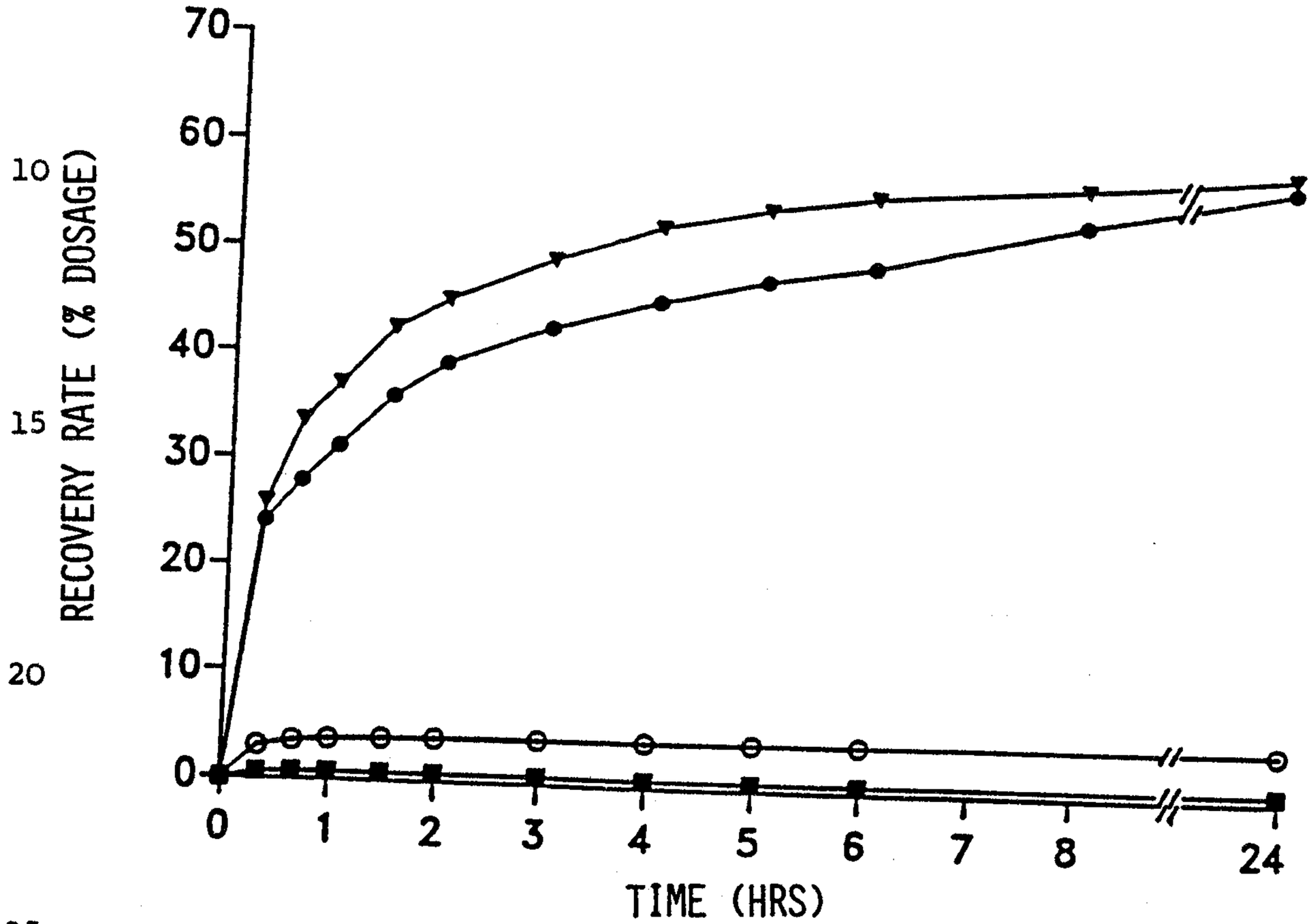
Figure 1 shows the amount of the administered dosage found in the lymph. For comparison there are quoted the values which were obtained with two low-molecular active substances whose distribution coefficient in octanol/water is $<10^{34}$.

Figure 2 shows the amounts of active substance A found in the lymph after administration in mixed micelles and as an oily formulation.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. The use of mixed micelles as carriers for the parenteral therapeutic administration of low-molecular active substances having a distribution coefficient in octanol/water of greater than 10^4, with fat-soluble vitamins being excluded.
2. The use according to claim 1 for active substances having a distribution coefficient in octanol/water of greater than 10^6 and a molecular weight below 5000.
3. The use according to claim 1 or 2, in which the active substance is an immunomodulator, a cytostatic, an antiinfective or a contrast agent.
4. The use according to claims 1-3 for interstitial application.
5. The use according to claim 4 for intramuscular, subcutaneous or intradermal application.
6. A mixed micelle solution containing 2 to 200 mg/ml of a mixture of a micelle former and a lipid ingredient and 0.1 to 100 mg/ml of an immunomodulator.
7. A mixed micelle solution according to claim 6 wherein the micelle former and the lipid ingredient are present in the mixture at a ratio varying between 0.1:1 to 2:1.
8. A mixed micelle solution according to claim 6 or 7, in which the immunomodulator is (all-E)-3,7-dimethyl-9-(2-trifluoromethyl)-6-(nonyloxy)phenyl-2,4,6,8-nonatetraenoic acid.

Figure 1



Cumulative amounts of active substance found in the drained lymph vessel after i.d. administration in mixed micelles.

▼ Active substance B (1 mg/ml)
• Active substance A (0.4 mg/ml)
o Diclofensin (1 mg/ml)
■ Diazepam (0.8 mg/ml)

Composition of the mixed micelles:

Soya lecithin (40 mM) and Na glycocholate 1:1 in 3.8% aqueous mannitol solution.

Injection volume: 1 ml

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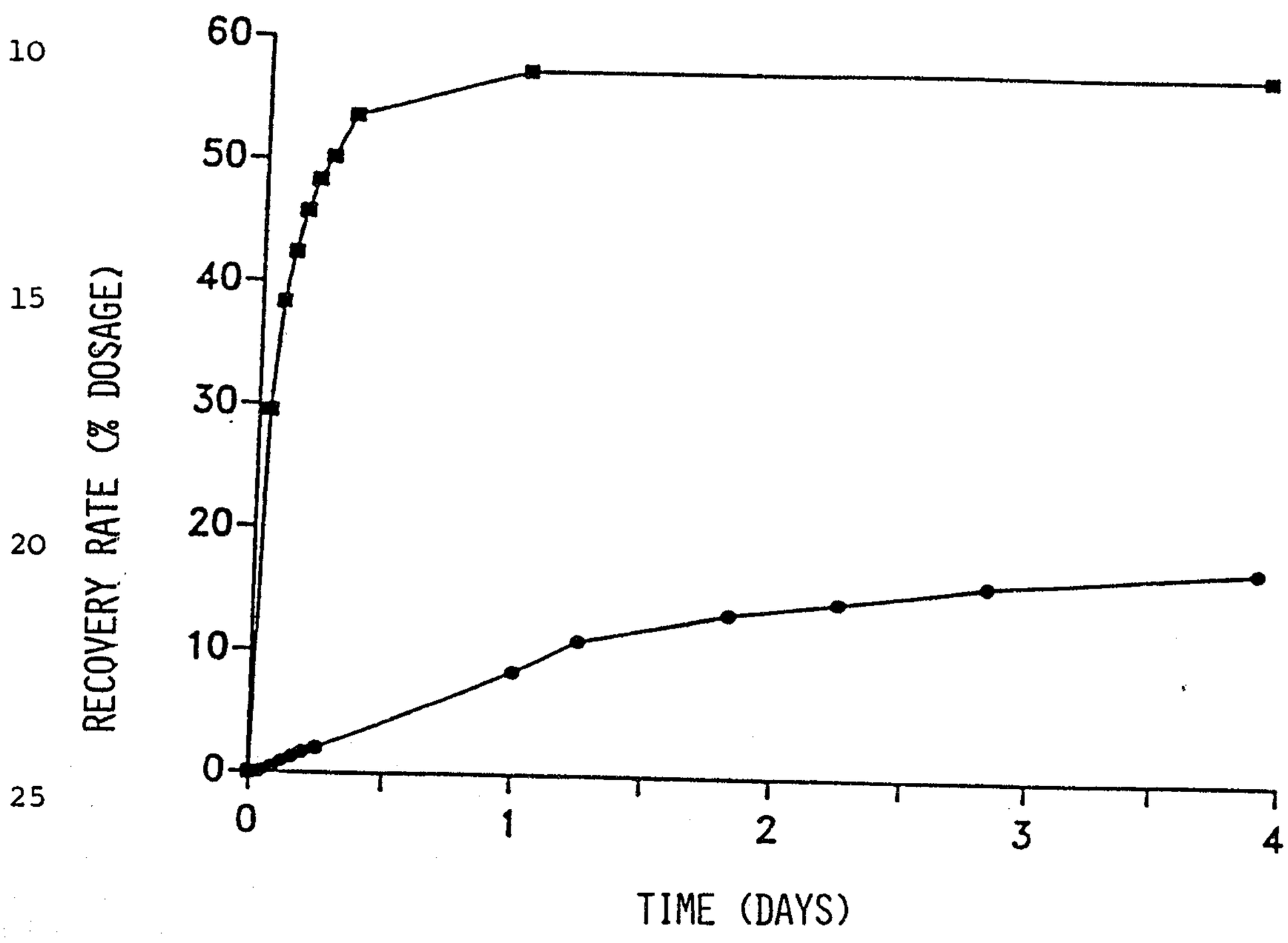
Figure 2



Cumulative amounts of active substance A found in the branching lymph vessel after i.d. administration

■ in mixed micelles

• in an oily formulation