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(54) Titre : COMPOSITIONS PHARMACEUTIQUES CONTENANT DES CYCLODEXTRINES ET DES TAXOIDES
(54) Title: PHARMACEUTICAL COMPOSITIONS CONTAINING CYCLODEXTRINS AND TAXOIDS

(57) Abrégé/Abstract:
A process for the preparation of rapidly soluble products for pharmaceutical use is provided. The process comprises the following steps: dissolving in ethanol a taxoid or a salt, or a hydrate, or a solvate thereof; adding to the solution a lyophilized solid cyclodextrin derivative; and evaporating the solvent and drying to obtain a solid, which comprises the taxoid as the active ingredient and the cyclodextrin derivative. The weight ratio of taxoid:cyclodextrin derivative ranges between 1:25 and 1:400. The taxoid is selected from the group consisting of paclitaxel and docetaxel, and the cyclodextrin derivative is selected from the group consisting of acetyl-γ-cyclodextrin and hydroxy-propyl-β-cyclodextrin. When hydroxy-propyl-β-cyclodextrin is used, the taxoid can be only docetaxel in a 1:25 to 1:100 taxoid:cyclodextrin weight ratio.

ABSTRACT

A process for the preparation of rapidly soluble products for pharmaceutical use is provided. The process comprises the following steps: dissolving in ethanol a taxoid or a salt, or a hydrate, or a solvate thereof; adding to the solution a lyophilized solid cyclodextrin derivative; and evaporating the solvent and drying to obtain a solid, which comprises the taxoid as the active ingredient and the cyclodextrin derivative. The weight ratio of taxoid:cyclodextrin derivative ranges between 1:25 and 1:400. The taxoid is selected from the group consisting of paclitaxel and docetaxel, and the cyclodextrin derivative is selected from the group consisting of acetyl-γ-cyclodextrin and hydroxy-propyl-β-cyclodextrin. When hydroxy-propyl-β-cyclodextrin is used, the taxoid can be only docetaxel in a 1:25 to 1:100 taxoid:cyclodextrin weight ratio.

PHARMACEUTICAL COMPOSITIONS CONTAINING CYCLODEXTRINS AND TAXOIDS

This invention relates to new water-soluble solid pharmaceutical compositions and their aqueous solutions for parenteral use containing taxoids and certain cyclodextrins without noxious solvents and/or detergents. More particularly the invention relates to new water-soluble solid pharmaceutical compositions and their solutions in aqueous solvents, the compositions containing

a) as an active ingredient a taxoid such as paclitaxel and docetaxel, optionally in the form of their salts or their polymorphic hydrates or solvates e.g. solvates formed with ethanol; and

b) a large surface area acetyl-γ-cyclodextrin or hydroxy-propyl-β-cyclodextrin (in the following the cyclodextrin)

- whereby the weight ratio of said taxoid to said cyclodextrin ranges between 1 : 25 and 1 : 250;

c) and optionally other water-soluble auxiliary materials usual in pharmaceuticals for parenteral purposes.

The invention also relates to the preparation and use of said injectable pharmaceutical compositions.

The following definitions are used throughout the specification and claims:

paclitaxel = taxol™ A;

docetaxel = (N-debenzoyl-N-tert.-butoxycarbonyl)-10-deacetyl-paclitaxel ;

"aqueous solvents" = water of injectable quality or an aqueous solution containing a dissolved isotonizer in an amount and concentration effective to isotonize said aqueous solution e.g. sodium chloride, glucose;

"amorphised" = showing amorphous structure against X-rays;

CD = cyclodextrin

γ-cyclodextrin = cyclomaltooctaose

β-cyclodextrin = cyclomaltoheptaose

Ac γCD = acetyl-γ-cyclodextrin

HPßCD = hydroxypropyl-ß-cyclodextrin (about 2 to about 8 hydroxypropyl groups per CD-unit)

DIMEß = heptakis-2,6-di-0-methyl-ß-cyclodextrin

Taxoid is the collective name for paclitaxel and structurally related substances. Paclitaxel is a compound known for its significant anticancer activity acting as a poison to mitotic spindles and as a potent inhibitor of cell replication. It also is known to have poor aqueous solubility of about 0.0005 mg/ml at ambient temperature and is reported to be unstable both in solution upon storage and as a consequence of solvolysis of its ester linkage leading to loss of cytotoxic activity. It is marketed in solution of a 1 : 1 mixture of ethanol and Cremophor™ EL (polyhydroxylated castor oil).

Cremophor EL has been implicated in some adverse reactions (hypersensitivity, histamine release) during intravenous treatment and therefore many attempts have been made in the past decade to develop new drug delivery systems.

When paclitaxel is formulated as a solution in the organic solvents ethanol and polyethoxylated castor oil, it is administered as a supersaturated oil-in-water emulsion and dilutes rapidly in the blood, an aqueous environment rich in hydrophobic domains provided by lipids and proteins. Paclitaxel passes through the hydrophobic environment of the plasma membrane while entering and leaving cells by diffusion.

The propensity of paclitaxel to undergo solvent- and concentration-dependent self-aggregation has hampered efforts to formulate this poorly soluble drug in pharmaceutically acceptable excipients for clinical administration.

It is also known that docetaxel, a semisynthetic taxoid is highly lipophylic and more water soluble than paclitaxel [USP 4814470]. Commercially available docetaxel for injection concentrates is a sterile solution of the drug in polysorbate

80, with an accompanying diluent of 13% (w/w) ethanol in water for injection. The maximal drug concentration which could be used was 0.3 to 0.9 mg/ml. Levels higher than 0.9 mg/ml had to be avoided because of precipitation issues. Hypersensitivity reactions associated with polysorbate 80 might cause problems with commercially available formulations

A wide range of approaches has been taken in formulation, including vehicles with hydrophobic characteristics. In most vehicles the taxoid is formulated in the millimolar concentration range which is comparable to the concentrations at which the taxoid was observed to self-aggregate resulting in precipitation.

Based on the interaction between the taxoid and cyclodextrins several further attempts at solving the problem were made. Studying the thin-layer chromatographic mobility of the taxoid in the presence of cyclodextrins (Int. J. Pharm. 1994. 108. 64-75.) it was first concluded that the interaction between taxoid and cyclodextrin was very weak. Later, a modest enhancement of the solubility of the taxoid was found using unsubstituted cylclodextrins and enzyme-modified branched-β-cyclodextrins.

Aqueous parenteral solutions of sparingly soluble drugs in water combined with cyclodextrins were suggested to minimize drug precipitation at injection sites or organs following parenteral application (US Patent No. 5,024,998 dated June 18, 1991). Solubility enhancement was hereby observed using high (around 50% w/v) CD concentration and solid complexes were obtained with some products from these concentrates. This emphasized that below a 20% w/v CD concentration precipitation might occur. Among the tremendous group of drugs listed in this specification, the document is silent on the use of taxoids and acetyl-YCD.

Using an aqueous solution of methylated β-cyclodextrins as effective paclitaxel solubility enhancers (PCT WO 94/26728) and combining of methylated β-cyclodextrin and ethanol for preparation of 1 to 4 mg/ml paclitaxel solutions (EP 788373) represented further improvements in this field. These taxoid con-

centrates did not precipitate on dilution up to a certain limit and avoided use of toxic detergents. Also the use of 2,6-di-O-methyl-β-cyclodextrin was suggested as a more effective solubilizer of paclitaxel (EPA 0639380) and detailed results on its solubilizing potency were published (J. Pharm. Sci. 84. 10. 1223-1230; 1995). However some concern was expressed concerning the use of methylated β-cyclodextrins in injectables because of their surfactant properties, their affinity to cholesterol and other cell-membrane lipid components already at low concentration and doses.

Some studies were published on the use of cyclodextrins along with water-soluble drugs to achieve less ulceration compared to the same formulation of cytotoxic compounds when extravasated (WO 95/06485). Though paclitaxel is not water-soluble, this patent specification also includes an example of "Taxol" in 50% ethanol solution to illustrate this effect adding "HPCD". The results show that HPCD exerted a protective effect on skin when Taxol was deposited into an intradermal site, resulting in the reduction in lesion size.

Thio-branched cyclodextrins were disclosed for solubilizing anticancer taxoids (e.g. docetaxel and paclitaxel see PCT WO 95/19994) without details concerning improvement of solubility, stability or toxicity. Such formulations provided a significant increase in aqueous solubility for paclitaxel. However on dilution for parenteral application (0.3 to 1.2 mg/ml) precipitation of the drug occured.

It was an aim of the present invention to further improve both solubility of taxoids in injectable formulations and stability of taxoid drug systems for parenteral administration. Moreover, possibly decreasing toxicity of accompanying materials used for formulation purposes.

The basis of the present invention is the recognition that both acetylated-γ-cyclodextrin and hydroxy-propyl-β-cyclodextrin exhibit a positive effect on the prolongation of the oversaturated state of dissolved paclitaxel and docetaxel, thus preventing premature precipitation without using ethanol as a

co-solvent. This was a surprising fact considering γ-cyclodextrin and its hydroxypropylated derivatives - which are known to possess the largest size cavities among the CDs - have been previously disclosed to be less suitable for paclitaxel solubilization and complexation (Int. J. Pharm. 133. 191-201.1996). This was confirmed by our studies registering the equilibrium solubility of paclitaxel as a function of the concentration of aqueous solutions of chemically modified γ-cyclodextrins whereby no practically useful solubilizing effect was found considering high cyclodextrin-concentrations were used. See **Table 1**: Solubility of paclitaxel as a function of acetyl-γ -cyclodextrin concentration).

Table 1

Acetyl-γ -cyclodextrin(%) w/v	**Dissolved Paclitaxel (µg/ml)**
2	4.0
5	8.8
10	25.0
20	48.0
40	170.0

The aqueous solubility of paclitaxel at room temperature is about 0.5 µg/ml, therefore the 170 µg/ml dissolved paclitaxel concentration achieved in 40% acetyl-γ -cyclodextrin solution already means an about 340-fold solubility enhancement. The above data however are poor as compared with the known results according to which in an aqueous 40% w/v solution of DIMEß dissolves about 800-1000 µg/ml of paclitaxel. Similar poor results were found using HPßCD as a solubilizer. This value is far from the desirable value corresponding to the therapeutic dose of paclitaxel and the use of such a dosage form would require administration of more than 70 grams of cyclodextrin with the paclitaxel dose unit of 30 mg.

An object of the present invention is a process for the preparation of pharmaceutical compositions for parenteral use in instantly water-soluble solid state as well as the solutions thereof in aqueous solvents containing acetyl-γ - cyclodextrin or hydroxy-propyl- β-cyclodextrin (in the following named cyclodextrin) comprising

a) dissolving a taxoid preferably paclitaxel or docetaxel, or their salts or hydrates in ethanol and thereafter performing either of the following steps:

i) adding the solid cyclodextrin and optionally other water-soluble auxiliary materials usual in pharmaceuticals for parenteral purposes, dissolving the mixture in an aqueous solvent and lyophilising to obtain a solid or

ii) adding the solid, large surface area amorphous (preferably lyophilised) cyclodextrin and optionally other water-soluble auxiliary materials usual in pharmaceuticals for parenteral purposes, evaporating the solvent and drying to obtain a solid or

iii) admixing the solution with solid large surface area amorphous preferably freeze-dried cyclodextrin (and optionally other water-soluble auxiliary materials usual in pharmaceuticals for parenteral purposes), thereafter dissolving with an aqueous solvent optionally in the presence of an effective amount of an isotonizing additive to obtain a solution;

containing

a) as an active ingredient the taxoid and

b) the cyclodextrin and

c) optionally other water-soluble auxiliary materials usual in pharmaceuticals for parenteral purposes

whereby the weight ratio of taxoid : cyclodextrin is between the range 1 : 25 to 1 : 400; and optionally

b) when steps i) or ii) were used dissolving the solid in an aqueous solvent

to obtain a parenteral solution ready for direct medical treatment.

According to the invention the taxoid paclitaxel is used in its hydrated polymorphic form or in its solvated form such as the ethanol solvate.

In a preferred embodiment of the invention the active ingredient paclitaxel is used together with acetyl-Y-cyclodextrin in a 1 : 100 to 1 : 250 weight ratio. In another preferred embodiment docetaxel is used with HPßCD in the range of 1 : 25 to 1 : 100 weight ratio.

When carrying out the process according to the invention any of the following can be used as the aqueous solvent or diluent: water of injectable quality, an aqueous solution of an isotonizing additive such as sodium chloride, glucose, mannitol, dextrose. The latter solutions have to contain the ingredients in an amount to effectively isotonize the aqueous solution under the conditions hereunder. Thus the concentrations known to be suitable for isotonizers in pharmaceutical solutions might be changed somewhat by the presence of the cyclodextrins and the taxoids employed. Thus concentrations in the magnitude of about 0.9 % w/v of sodium chloride, or about 5% w/v of glucose have to be optimised for the specific composition depending on the taxoid employed and on the amount and quality of the CD used.

A further object of the present invention is to provide pharmaceutical compositions containing as active ingredients any of the products prepared according to the processes of the invention.

An additional object of the present invention is to provide water-soluble solid pharmaceutical compositions and

their solutions in aqueous solvents, the compositions containing

a) as an active ingredient a finely dispersed large surface area taxoid such as paclitaxel or docetaxel, optionally in the form of their salts or their polymorphic hydrates or solvates e.g. formed with ethanol
and

b) finely dispersed, large surface area acetyl-γ -cyclodextrin or hydroxy-propyl-β-cyclodextrin (cyclodextrin)

the weight ratio of said taxoid : said cyclodextrin ranging between 1 : 25 and 1 : 250;

c) and optionally other water-soluble auxiliary materials usual in pharmaceuticals for parenteral purposes.

Preferred compositions according to the invention contain as the active ingredient paclitaxel and acetyl-Y-cyclodextrin as the cyclodextrin in a 1 : 100 to 1 : 250 weight ratio. Further preferred compositions are combinations of docetaxel and hydroxy-propyl-β-cyclodextrin in a 1 : 25 to 1 : 100 weight ratio.

A further object of the present invention is to provide methods for prevention of self-aggregation and premature precipitation of a taxoid such as paclitaxel and docetaxel and their salts, solvates and hydrates in aqueous solutions and for prolongation of the oversaturated dissolved state of the drugs. This is accomplished by using the taxoids in the form of pharmaceutical compositions according to the present invention as described above in detail.

Yet another object of the present invention is to provide a method of treatment of unwanted cell proliferation by utilising effective amounts of pharmaceutical compositions

according to the invention as described above in detail. The ready-to-use paclitaxel solutions remain physically stable for a reasonable period of time. Intravenous treatment generally requires that solutions be used which are physically stable for at least 6 hours. When dissolving the compounds according to the present invention in aqueous solutions the thus reconstituted solutions are stable for at least 6 to 8 hours. In the case of solutions of the combinations paclitaxel /Acγ CD as well as docetaxel/AcγCD or HPβCD more than 24 hours of physical stabilities were measured.

According to the method it is provided to administer to a patient in need of such treatment a parenteral dosage form containing the pharmaceutical composition. Dosage units of 100 mg taxoid may be prepared in lyophilised form. These are diluted to give the ready to use dosage solutions applicable e.g. for intravenous treatment. Depending on the required treatment applicable in the specific needs of the patient several 100 mg units may be applied so as to reach the effective dose corresponding to the desired amount expressed generally in mg/m^2 body surface. Thus e.g. the recommended dose of paclitaxel ranging from 135 to 250 mg/m^2 body surface can be reached. The drug can be used effectively by way of intravenous, intraperitoneal, intramuscular administration depending on the type of cancer to be treated and the taxoid and cyclodextrin selected for use. Improved results of treatment as compared with known therapies can be achieved due to the considerably decreased toxicity of the dosage form as compared with the toxicity of the vehicles contained in the known compositions used to date. It is also not necessary to subject the patient to premedication against hypersensitivity and other unwanted side-effects using steroids, antihistamines and H_2-receptor antagonists before paclitaxel or docetaxel administration to prevent severe hypersensitivity reactions.

BRIEF DESCRIPTION OF THE DRAWINGS

Exemplary embodiments of the present invention will now be described in conjunction with the following drawings, in which:

Figure 1 shows arterial blood pressure values systolic (spheres) and diastolic (triangles) after administration of Cremophor/Ethanol mixture without paclitaxel intravenously.

Figure 2 shows arterial blood pressure values systolic (spheres) and diastolic (triangles) after administration of Cremophor/Ethanol mixture intravenously.

Figure 3 shows arterial blood pressure values systolic (spheres) and diastolic (triangles) after administration of paclitaxel/Acetyl-Y-cyclodextrin in 10, 20, 40, 60 mg/m^2 dose according to Example I dissolved in 2.5% aqueous dextrose solution intravenously.

Figure 4 shows arterial blood pressure values systolic (spheres) and diastolic (triangles) after administration of paclitaxel/Acetyl-Y-cyclodextrin in 120, 90, 240 mg/m^2 dose according to Example I dissolved in 2.5% aqueous dextrose solution intravenously.

Figure 5 shows arterial blood pressure values systolic (spheres) and diastolic (triangles) after administration of paclitaxel/Acetyl-Y-cyclodextrin in 10, 90, 40, 60 mg/m^2 dose according to Example I dissolved in 2.5% aqueous dextrose solution intravenously.

Figure 6 shows arterial blood pressure values systolic (spheres) and diastolic (triangles) after administration of paclitaxel/Acetyl-Y-cyclodextrin in 120, 90, 240 mg/m^2 dose according to Example I dissolved in 2.5% aqueous dextrose solution intravenously.

In Figures 1 to 6, the arrow marks the time when the injection was given.

Details of the invention are given in the Examples below by way of illustration and without the intention to limit the scope of protection.

I. PREPARATION OF FORMULATIONS

Example I.1.: Preparation of reconstitutable, solid paclitaxel /acetyl-γ-cyclodextrin combinations by drying.

3 mg of paclitaxel (dissolved in 0.3 ml of ethanol) was added to 500 mg of acetyl-γ-cyclodextrin powder (freeze-dried from aqueous solution). This mixture was kept under vacuum for 18 hours over phosphorous pentoxide to remove the ethanol. Portions of this ethanol-free powder combination were dissolved in 5, 4, 3 and 2 ml of 5% aqueous glucose solutions respectively. The physical stability of these reconstituted solutions is shown in Table 2.

TABLE 2

	Added ml of 5% glucose solution			
	5 ml	4 ml	3 ml	2 ml
final paclitaxel mg/ml	0.6	0.75	1.0	1.5
final AcγCD mg/ml	100	125	167	250
stability evaluated visually in hours.	>120	> 48 after 72 hours precipitation occurred	> 48	> 48
dissolved paclitaxel mg/ml (HPLC)	0.576			

This experiment was repeated with lower amounts of acetyl-γ-cyclodextrin. 3 mg of paclitaxel (dissolved in 0.3 ml of ethanol) was added to 450 mg, 400 mg or 350 mg of freeze-dried acetyl-γ -cyclodextrin samples, thereafter the combination was kept under vacuum for 18 hours over phosphorous pentoxide to remove the ethanol.

These combinations were dissolved in 5, 4, 3 and 2 ml each of an 5% aqueous glucose solution. In Table 3, 4, 5

the composition as well as the physical stability of these reconstituted paclitaxel solutions prepared according to the present example using different amounts of acetyl-Y- cyclodextrin are summarised.

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TABLE 3

	Added ml of 5% glucose solution			
	5 ml	4 ml	3 ml	2 ml
final paclitaxel mg/ml	0.6	0.75	1.0	1.5
final acetyl- γ- cyclodextrin	90	112.5	150	225
stability, evaluated visually in hours.	opalescent solution	> 48	> 48	> 48

TABLE 4

	Added ml of 5% glucose solution			
	5 ml	4 ml	3 ml	2 ml
final paclitaxel mg/ml	0.6	0.75	1.0	1.5
final acetyl γCD mg/ml	80	100	133	200
stability, evaluated visually in hours.	< 4 slight opalescence			> 24 clear solution
dissolved paclitaxel mg/ml on prep. after 22 hours.	 0.41 0.42			 1.26 1.25

TABLE 5

	Added ml of 5% glucose solution			
	5 ml	4 ml	3 ml	2 ml
final paclitaxel mg/ml	0.6	0.75	1.0	1.5
final Ac γCD	70	86	117	175
stability, evaluated visually in hours.		increasing opalescence	increasing opalescence	> 24 almost clear solution
dissolved paclitaxel mg/ml (HPLC)				

at prep.				1.16
after 22 hours.				1.17

Example I.2.: Preparation of a paclitaxel formulation of 0.6 mg/ml nominal paclitaxel concentration

6.5 g of amorphised acetyl-Y-cyclodextrin was wetted with 3 ml of a paclitaxel solution of 10 mg/ml paclitaxel concentration (corresponding to 30 mg of paclitaxel). Immediately 50 ml of a 5% dextrose solution was added to the wet mixture and the composition was agitated until a clear solution was obtained. The dissolved paclitaxel concentration in solution amounted to 0.55 ± 0.05 mg/ml as determined by HPLC after filtration of the solution through a 0.2 µm membrane filter. The solution was then stored at room temperature under normal light conditions in glass containers for at least 6 hours without noticeable opalescence and/or particle formation. The re-analysis of the dissolved paclitaxel after 6 hours of storage by HPLC showed that no decrease of dissolved paclitaxel concentration was apparent.

Example I.3.: Preparation of a paclitaxel formulation of ~ 0.3 mg/ml nominal paclitaxel concentration

6.5 g of previously amorphised acetyl-γ-cyclodextrin was wetted with 3 ml of a paclitaxel solution of 10 mg/ml paclitaxel concentration (corresponding to 30 mg of paclitaxel). To this wet mixture 100 ml of a 5% dextrose solution was immediately added and the mixture was agitated until a clear solution was obtained. The dissolved paclitaxel concentration in this solution amounted to 0.27 ± 0.03 mg/ml, as determined by HPLC after filtration of the solution across a 0.2 µm membrane filter. The solution was then stored at room temperature under normal light conditions in glass containers for at least 12 hours without noticeable opalescence and/or particle formation. The HPLC re-analysis of the dissolved paclitaxel after 12 hours of storage showed that no decrease of dissolved paclitaxel concentration resulted during the 12 hours.

Example I.4.: Preparation of docetaxel formulation of 0.5 and 1 mg/ml nominal docetaxel concentration

100 mg of amorphised acetyl-γ -cyclodextrin was wetted with 0.1 ml of a docetaxel solution of 20 mg/ml docetaxel concentration (this corresponds to 2 mg of docetaxel). To samples of this wet solid mixture 1 or 2 ml of a 5% w/v aqueous dextrose solution was immediately added and the mixture was agitated until a clear solution was obtained. The dissolved docetaxel concentrations in solution according to the present example amounted to 1 and 0.5 mg/ml respectively. The solutions were then stored at room temperature under normal light conditions in glass containers for at least 24 hours without noticeable opalescence and/or particle formation. The composition of both solutions referred to 20 mg docetaxel dosage unit is as follows:

- 1000 mg AcγCD
- 20 mg docetaxel
- 1 ml ethanol
- 20 ml or 40 ml 5% dextrose

Example I.5.: Preparation of a docetaxel formulation of 0.75 mg/ml nominal docetaxel concentration using hydroxypropyl-β-cyclodextrin (HPβCD)

200 mg of HPβCD was wetted with 0.15 ml of a docetaxel solution of 10 mg/ml docetaxel concentration (This corresponds to 1.5 mg of docetaxel). To this wet mixture 2 ml of a 5% aqueous dextrose solution was immediately added and the mixture was agitated until a clear solution was obtained. The dissolved docetaxel concentration in solution according to the present example amounted to 0.75 mg/ml. The solutions were then stored at room temperature under normal light conditions in glass containers for at least 24 hours without noticeable opalescence and/or particle formation. The composition of solutions referred to 20 mg docetaxel dosage unit is as follows:

- 2670 mg HPβCD
- 20 mg docetaxel

- 2 ml ethanol
- 27 ml 5% dextrose

Example I.6.: Preparation of binary paclitaxel /acetyl-γ -cyclodextrin co-lyophilisate.

30 mg of paclitaxel dissolved in 3 ml of ethanol was added tc 5.0 g of amorphised acetyl-γ-cyclodextrin. To the wet powder an additional 2 ml of ethanol was added and the powder was dissolved in 50 ml of distilled water resulting in a clear solution with 0.6 mg/ml nominal paclitaxel concentration. This solution was frozen quickly on dry-ice and freeze dried to obtain a white lyophilisate with 0.6 ± 0.05 % / w paclitaxel content.

This lyophilisate was readily reconstituted into the original volume solution by way of adding 50 ml of a 5% aqueous glucose solution. The result was a clear solution with 0.6 mg/ml paclitaxel concentration.

The reconstituted solution was stored at room temperature under normal conditions in glass containers. The paclitaxel concentration was assayed using the HPLC method after filtration of the test solutions through a 0.2 µm membrane. The paclitaxel content

after preparation:	0.513 ± 0.07 mg/ml*
after 25 hours of storage	0.556 ± 0.01 mg/ml

(* The difference between nominal and measured paclitaxel concentration is due to the volume expansion of the bulk lyophilisate.)

Example I.7.: Preparation of binary docetaxel/acetyl-γ -cyclodextrin co-lyophilisate.

10 mg of docetaxel dissolved in 0.5 ml of ethanol was added to 400 mg of amorphised acetyl γ -cyclodextrin. The wet powder was dissolved in 8 ml of distilled water resulting in a clear solution with 1.25 mg/ml nominal docetaxel concentration. This solution was frozen quickly on dry-ice and freeze dried to obtain a white lyophilisate with 2.25 ± 0.1% docetaxel content.

50 mg of the solid lyophilisate was readily dissolved in 1 ml of a 5% aqueous glucose solution resulting in a clear solution of 1.13 mg/ml docetaxel content (determined by HPLC).

The reconstituted solution was then stored at room temperature under normal conditions in a glass container. After 21 hours of storage 1.15 ± 0.02 mg/ml docetaxel concentration was assayed using the HPLC method. Even after 72 hours of storage there was no visually observable opalescence or solid particle formation in the solution.

Example I.8.: Preparation of paclitaxel /acetyl-γ -cyclodextrin /glucose co-lyophilisate

30 mg of paclitaxel was dissolved in 3 ml of ethanol and the solution was then added to 5.0 g of amorphised acetyl-Y-cyclodextrin. To the wet powder an additional 2 ml of ethanol was added and the powder was then dissolved in 50 ml of a 5% aqueous glucose solution. This solution was frozen on dry-ice and freeze-dried to give a white lyophilisate with 0.42 ± 0.01 % paclitaxel content.

This lyophilisate was readily reconstituted into the original volume with the addition of 50 ml of distilled water. The result was a clear solution with 0.6 mg/ml nominal paclitaxel concentration.

The reconstituted solutions were then stored at room temperature under normal conditions in closed glass containers. The paclitaxel concentration during storage was measured using the HPLC method after filtration of the test solutions.

Paclitaxel content found:

immediately on preparation:	0.530 ± 0.08 mg/ml*
after 25 hours of storage	0.541 ± 0.05 mg/ml

(* The difference between nominal and measured paclitaxel concentration is due to the volume expansion of the bulk lyophilisate.)

Example I.9.: Preparation of docetaxel/acetyl-γ -cyclodextrin/glucose co-lyophilisate

8 mg of docetaxel dissolved in 0.4 ml of ethanol was added to 400 mg of amorphised acetyl-γ -cyclodextrin. The wet

powder was then dissolved in 8 ml of a 5% w/v aqueous glucose solution. This resulted in a clear solution with 1 mg/ml nominal docetaxel concentration which was frozen on dry-ice and freeze-dried, to give a white lyophilisate with 1% docetaxel content.

100 mg of ethanol free solid lyophilisate was readily reconstituted in 1, 2 or 4 ml of distilled water producing clear solutions of 1, 0.5 or 0.25 mg/ml nominal docetaxel concentrations, respectively. In Table 6, the physical stability of docetaxel solutions during 21 hours of storage at room temperature in closed glass containers are summarised. Determination of the dissolved docetaxel concentration at different nominal concentrations was made by HPLC.

TABLE 6

Dissolved docetaxel mg/ml HPLC	Nominal dissolved docetaxel concentration		
	1 mg/ml	0.5 mg/ml	0.25 mg/ml
at preparation	0.93	0.49	0.24
after 21 hours	0.89	0.48	0.24

Example I.10.:

The formulation according to Example I. 10. was prepared as step-wise described below:

1. 6.0 mg of crystalline Docetaxel (purity is 99.7%) was dissolved at room temperature in 3 milliliters (ml) of 96% ethyl alcohol.

2. This ethanolic docetaxel solution was dropped onto the surface of 300 mg of amorphous 2-hydroxypropyl-β-cyclodextrin (HPβCD) powder. The pre-mix was homogenised by a solid-state grinder for 5 minutes to ensure homogeneous distribution of dissolved Docetaxel on the surface of powder HPβCD.

3. The HPβCD powder previously wetted by the 3 ml of ethanolic Docetaxel solution was then dissolved in 6 ml of distilled water at room temperature by stirring with 300 r.p.m. using normal stirrer. (The resulting solution appears a clear, colorless, transparent solution with no visible solid undissolved particles. The dissolved Docetaxel drug concentration is 1 mg/ml.)

4. The above aqueous Docetaxel solution is chilled to minus 50 °C using dry ice/ethanol mixture freezing media, and then the solvent is removed by liophilisation. The freeze-dried (liophilised) product is a white, fluffy amorphous powder with a Doceteaxel content of 2% by weight.

This product is a water-soluble Docetaxel formulation: 50 mg of this formulation can be dissolved in 1 ml of water or in 1 ml 5% isotonic glucose solution, resulting in a clear, transparent Docetaxel solution with a Docetaxel concentration of 0.97 mg/ml. Moreover, this solution remains physically stable for over 21 hours without any Docetaxel precipitation upon standing at room temperature on daylight is glass vials.

Example I.11: Prolongation of the oversaturated dissolved state of the paclitaxel by way of co-evaporation

30 mg of Paclitaxel was dissolved in 6 ml of ethanol and the solution was mixed thoroughly with 5 g of acetyl-γ-cyclodextrin. An additional 3 ml of ethanol was added and the mixture was stirred for 5 minutes at room temperature. The ethanol content of the mixture was then removed in a nitrogen stream using a rotatory evaporator in vacuum. The resulting white solid was then dissolved in 5 ml of deionised water resulting in a slightly opalescent solution that was immediately frozen on dry-ice and freeze-dried. The resulting lyophilisate was an instantly soluble amorphous powder. This was conveniently reconstituted to a clear solution by adding injectable water or a 5% aqueous glucose solution. The reconstituted solution with 0.3 - 0.6 mg/ml dissolved paclitaxel concentration remained stable for at least 24 hours at room temperature.

Example I.12: Parenteral composition for intravenous use

100 mg of paclitaxel dissolved in 10 ml of ethanol was added to 15.0 g of amorphised large surface area acetyl-γ-cyclodextrin. To the wet powder an additional 10 ml of ethanol was added and the powder was dissolved in 80 ml of distilled water resulting in a clear solution with 1.25 mg/ml nominal paclitaxel concentration. This solution was frozen quickly on dry-ice and freeze-dried to yield a white lyophilisate with 0.7 ± 0.05 %w paclitaxel content.

This lyophilisate was readily reconstituted to 100 ml using a 5% w/v aqueous dextrose solution. A clear solution with 1.0 mg/ml paclitaxel concentration was obtained which was ready to use for intravenous application.

II. BIOLOGICAL STUDIES

The aim of these studies was to evaluate some specific side effects of intravenous administration of paclitaxel.

Cardiovascular and respiratory effects of paclitaxel following intravenous administration were examined on a total of 7 beagle dogs maintained under α-chloralose/sodium pentobarbitone anaesthesia.

II.1. TEST SUBSTANCES

A. Paclitaxel 1 ml liquid (30 mg/ml in ethanol) supplied in vials. Dissolved in the vehicle Cremophor ELP : ethanol 1:1 on the course of the test.

B. Paclitaxel/AcyCD white crystalline powder; prepared according to the above-identified Example I. 1.6. Dissolved in vehicle 2.5% w/v aqueous dextrose on the course of the test.

II.2. METHODS

The following parameters were recorded or derived and served as indicators of the functional status of the various elements within the cardiovascular and respiratory systems. The units of measurement are stated in parenthesis.

Systolic, diastolic, mean blood pressure (mmHg) Heart rate (beats/minute)	General haemodynamic status
Left ventricular systolic pressure (mmHg) Left ventricular dp/dt max. (mmHg. sec^{-1})	Contractile status of the myocardium
Electrodiagram (lead H)	Electrical status of the myocardium
Femoral flow (ml/minute) Femoral resistance (mmHg/ml/minute)	Status and resistance of the peripheral vasculature
Respiration rate (breaths/minute) Respiration minute volume (ml) Respiration tidal volume (ml)	General respiratory status

Anaesthesia was initiated by i.v. injection of sodium thiopentone and maintained through an i.v. with a mixture of α-chloralose and sodium pentobarbitone given as required. Body temperature was maintained at a constant level.

The trachea was cannulated and the canule connected to a pneumotach screen to allow measurement of tidal volume (ml) and the derived parameters of respiration rate (br/min) and minute volume (ml). Catheters were introduced into the right femoral artery and a cephalic vein to facilitate measurements of blood pressure and the administration of vehicle, test substances and anaesthetic respectively. The femoral artery was connected to a heparin/saline filled pressure transducer, coupled to a Grass 7E polygraph. Heart rate was derived elec-

tronically from the blood pressure signal.

An ultrasonic flow probe was positioned around the left femoral artery and connected to a blood flow meter coupled to the Grass polygraph to record femoral blood flow (ml/min).

A catheter was introduced into the left carotid artery and advanced into the left ventricle to facilitate the measurement of the left ventricular systolic pressure (LVSP). The catheter was connected to a heparin/saline-filled pressure transducer and the LVSP signal was displayed on the Grass polygraph.

Subcutaneous needle electrodes were inserted in the appropriate limbs in order to monitor the electrocardiogram which was displayed on the Grass polygraph. Various signals were transferred from the Grass polygraph to a computer and the captured data was analysed and displayed.

Following a 30 minute stabilization period, the vehicle was administered through an i.v. at a dose volume of 7.0 ml/kg over a period of 15 minutes. Forty-five minutes later and a minimum of 45 minutes intervals thereafter doses of the drugs were administered through an i.v. at a dose volume of 7.0 ml/kg again over 15 minutes each. The parameters were monitored for at least 45 minutes from the start of infusion. All doses were administered using a KdS model 200 infusion pump.

Following samples were tested:

N°	Animal	Vehicle i.v. 7 ml/kG, 15 min.	Drug	Drug Dose mg/m^2
1.		Cremophor ELP : ethanol	paclitaxel	6; 60
2.		Cremophor ELP : ethanol	paclitaxel	6; 60
3.	2901AK	Cremophor ELP : ethanol	none	-
4.	2841AK	Cremophor ELP : ethanol	none	-
5.	D2515	2.5% dextrose/ in water	paclitaxel/AcγCD*	10; 20; 40; 60
6.	D2937	2.5% dextrose/ in water	paclitaxel/AcγCD*	40, 10, 60, 90
7.	D5	2.5% dextrose/ in water	paclitaxel/AcγCD*	90; 120; 240

*= prepared according to Example I.6.

All parameters including high resolution (50 mm/sec) recordings of the ECG were measured at 5 minute intervals during the stabilization period and at 0.5, 1, 2 and 5 minute inter-

vals post-dose following vehicle and drug administration. At the end of the full observation period the animals were sacrificed with an overdose of sodium pentobarbitone through the i.v.

II.3. RESULTS

Animals	Results
1 and 2	Administration of 6 and 60 mg/m^2 paclitaxel in Cremophor ELP : ethanol 1:1 was discontinued because of premature death of the animals.
3 and 4	Intravenous administration of Cremophor ELP : ethanol at both dose levels (corresponding to the doses used with 6 and 60 mg/m^2 paclitaxel respectively) induced an anaphylactic type response: immediate decrease in arterial blood pressure, heart rate, left ventricular systolic pressure and left ventricular dp/dt maximum of similar magnitude in both animals. Little or no recovery in any cardiovascular parameter was observed post-dose. Both doses levels induced ventricular ectopic beats, reduced the overall amplitude of the ECG wave-form, caused notching of the T-wave and reduction in T-wave amplitude. In animal 2841AK the vehicle also induced elevation of the S-T segment. As a result of these finding it may be concluded that the solvent-mixture per se is toxic and should be avoided as an i.v. administration.
5; 6; 7	Administration of 2.5% w/v of dextrose at a dose volume of 7 ml/kg induced no overall overt effects on any of the cardiovascular or respiratory parameters in the 3 animals tested. Addition of the paclitaxel/AcγCD doses up to 240 mg/m2 in general caused only small changes in the measured cardiovascular and respiratory parameters. These small changes included slight increases in arterial blood pressure and left ventricular systolic pressure, accompanied by decreases in heart rate and left ventricular dp/dt maximum. Other inconsistent changes included increases in femoral flow, tidal volume and respiration rate. These findings were reversible.

II.4. FIGURES

To illustrate an important parameter of the results the effect on arterial blood pressure following i.v. administration in the anaesthetised beagle dog is shown in Figures 1 to 6:

Arterial blood pressure (mmHg) versus time (minutes):

-●-●-●- systolic -▲-▲-▲- diastolic

Graph	animal	vehicle	drug mg/m^2
Figure 1	2901AK	Cremophor : ethanol	none
Figure 2	2841AK	Cremophor : ethanol	none
Figure 3	D2515	2.5% dextrose in water	paclitaxel/AcγCD Ex. I. 6. 10, 20, 40, 60
Figure 4	D5	2.5% dextrose in water	paclitaxel/AcγCD Ex. I. 6. 120, 90, 240
Figure 5	D2937	2.5% dextrose in water	paclitaxel/AcγCD Ex. I .6 10, 90; 40; 60
Figure 6 mean pressure	D5	2.5% dextrose in water	paclitaxel/AcγCD Ex. I. 6 120; 90; 240

II.5. SUMMARY:

Intravenous administration of the Cremophor ELP : ethanol 1:1 vehicle as well as paclitaxel along with this vehicle caused an anaphylactic type reaction and is thus inadequate to be administered. Administration of paclitaxel/acetyl-γ-CD (from 20 mg up to 240 mg/m^2) in aqueous dextrose as vehicle failed to produce marked and persistent cardiovascular overt effects on any of the parameters in any of the animals tested.

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

1. A process for the preparation of rapidly water-soluble products for pharmaceutical use comprising the steps of:

a) forming a solution by dissolving in ethanol a taxoid selected from the group consisting of paclitaxel, docetaxel, a salt thereof, a hydrate thereof, and a solvate thereof,

b) adding to the solution a lyophilized solid cyclodextrin derivative selected from the group consisting of acetyl-γ-cyclodextrin and hydroxy-propyl-β-cyclodextrin, and

c) evaporating the ethanol and drying to obtain a solid comprising:

A. the taxoid as the active ingredient, and

B. the cyclodextrin derivative,

whereby the weight ratio of taxoid:cyclodextrin derivative ranges between 1:25 and 1:400, and whereby when hydroxy-propyl-β-cyclodextrin is used, the taxoid can be only docetaxel in a 1:25 to 1:100 taxoid :cyclodextrin weight ratio.

2. A process for the preparation of a solution in water of rapidly water-soluble solid products for pharmaceutical use comprising the steps of:

a) forming the solution by dissolving in ethanol a taxoid selected from the group consisting of paclitaxel, docetaxel, a salt thereof, a hydrate thereof, and a solvate thereof, and thereafter performing any one of the following steps:

i) adding to the solution a solid cyclodextrin derivative selected from the group consisting of acetyl-γ-cyclodextrin and hydroxy-propyl-β-cyclodextrin, dissolving the mixture in an aqueous solvent, and lyophilizing the resulting solution to obtain a rapidly soluble solid; or

ii) adding to the solution a lyophilized solid cyclodextrin derivative selected from the group consisting of acetyl-γ-cyclodextrin and hydroxy-propyl-β-cyclodextrin, evaporating the ethanol and drying to obtain the rapidly soluble solid; and

b) dissolving the rapidly soluble solid in water thus obtaining the solution for direct medical treatment comprising:

A. the taxoid as the active ingredient, and

B. the cyclodextrin derivative,

wherein the weight ratio of taxoid:cyclodextrin ranges between 1:25 and 1:400, and whereby when hydroxy-propyl-β-cyclodextrin is used, the taxoid can be only docetaxel in a 1:25 to 1:100 taxoid :cyclodextrin weight ratio.

3. A process for the preparation of rapidly water-soluble solid products for pharmaceutical parenteral use comprising the steps of:

a) dissolving in ethanol a taxoid selected from the group consisting of paclitaxel, docetaxel, a salt thereof, a hydrate thereof and a solvate thereof to form a solution,

b) adding to the solution a lyophilized solid cyclodextrin derivative selected from the group

consisting of acetyl-γ-cyclodextrin and hydroxy-propyl-β-cyclodextrin,

c) evaporating the ethanol and drying to obtain an rapidly soluble solid,

d) dissolving said solid in an aqueous solvent thus obtaining an aqueous solution, and

e) freezing this solution and lyophilizing it to obtain an rapidly soluble lyophilizate comprising:

A. the taxoid as the active ingredient, and

B. the cyclodextrin derivative,

wherein the weight ratio of taxoid:cyclodextrin ranges between 1:25 and 1:400, and whereby when hydroxy-propyl-β-cyclodextrin is used, the taxoid can be only docetaxel in a 1:25 to 1:100 taxoid :cyclodextrin weight ratio.

4. A process for the preparation of a solution in water of rapidly water-soluble solid products for pharmaceutical parenteral use comprising the steps of:

a) dissolving in ethanol a taxoid selected from the group consisting of paclitaxel, docetaxel, a salt thereof, a hydrate thereof, and a solvate thereof to form the solution,

b) adding to the solution a lyophilized solid cyclodextrin derivative selected from the group consisting of acetyl-γ-cyclodextrin and hydroxy-propyl-β-cyclodextrin,

c) evaporating the solvent and drying to obtain an rapidly soluble solid,

d) dissolving said solid in an aqueous solvent thus obtaining an aqueous solution,

e) freezing the aqueous solution and lyophilizing it to obtain a rapidly soluble solid lyophilizate, and

f) re-dissolving the lyophilizate in water thus obtaining the solution comprising:

A. the taxoid as the active ingredient, and

B. the cyclodextrin derivative,

wherein the weight ratio of taxoid:cyclodextrin derivative ranges between 1:25 and 1:400, and whereby when hydroxy-propyl-β-cyclodextrin is used, the taxoid can be only docetaxel in a 1:25 to 1:100 taxoid :cyclodextrin weight ratio.

5. The process for the preparation of solutions according to claim 2 or 4, wherein the aqueous solvent comprises: injectable quality water or a water solution, the solution comprising an isotonizing additive, wherein the isotonizing additive is sodium chloride, glucose or dextrose in an amount capable of isotonizing the aqueous solution.

6. The process for the preparation of solid products according to claim 1 or 3, wherein the cyclodextrin derivative comprises: acetyl-Y-cyclodextrin having a degree of acetylation between 2 to 12 acetyl groups per cyclodextrin ring, or hydroxy-propyl-β-cyclodextrin having a degree of substitution between 2 to 10 hydroxypropyl groups per cyclodextrin ring.

7. The process for the preparation of solid products according to claim 1 or 3, wherein the cyclodextrin derivative comprises: acetyl-Y-cyclodextrin having a

degree of acetylation of 8 acetyl groups per cyclodextrin ring.

8. The process for the preparation of solutions according to claim 2 or 4, wherein the cyclodextrin derivative comprises: hydroxy-propyl-β-cyclodextrin having a degree of substitution between 4 to 6 hydroxypropyl groups per cyclodextrin ring.

9. The process for the preparation of solid products according to claim 1 or 3, wherein the paclitaxel is used in its hydrated form or in its ethanol solvate form.

10. Rapidly soluble solid products for pharmaceutical use prepared by the process according to any one of claims 1, 3, 6, 7, and 9.

11. Solutions of a solid product prepared according to claim 1 or 3 in aqueous solvents.

12. Pharmaceutical compositions comprising a solid product prepared by the process according to any one of claims 1, 3, 6, 7, and 9.

13. Use of the solid product prepared by the process according to any one of claims 1, 3, 6, 7, and 9 for the treatment of cancer.

14. Pharmaceutical compositions comprising a solution prepared by the process according to any one of claims 2, 4, 5, and 8.

15. Use of the solution prepared by the process according to any one of claims 2, 4, 5, and 8 for the treatment of cancer.

FIGURE 1

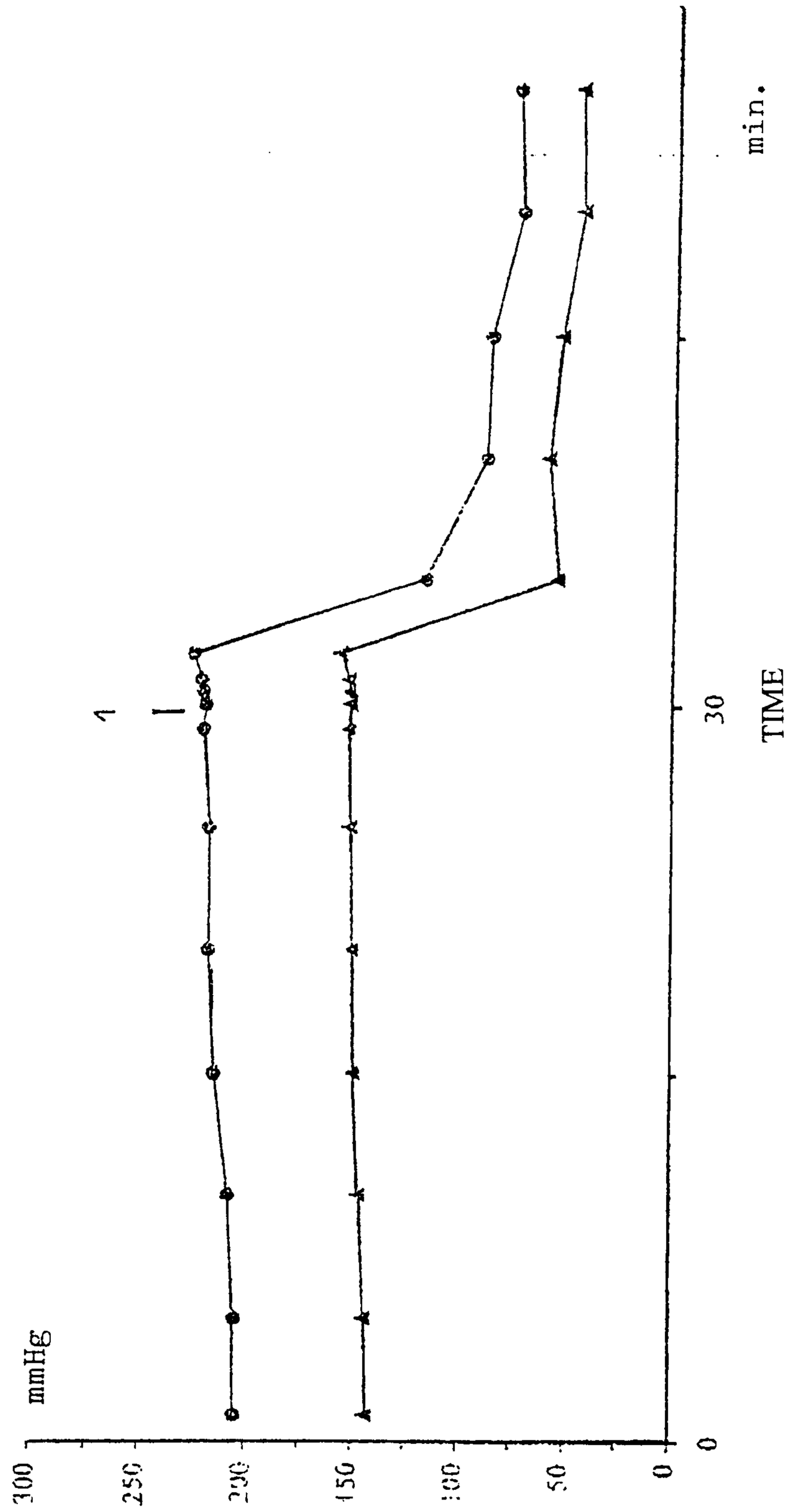

ARTERIAL BLOOD PRESSURE
mmHg
300
250
200
150
100
50
0
1
30
TIME
min.

FIGURE 2

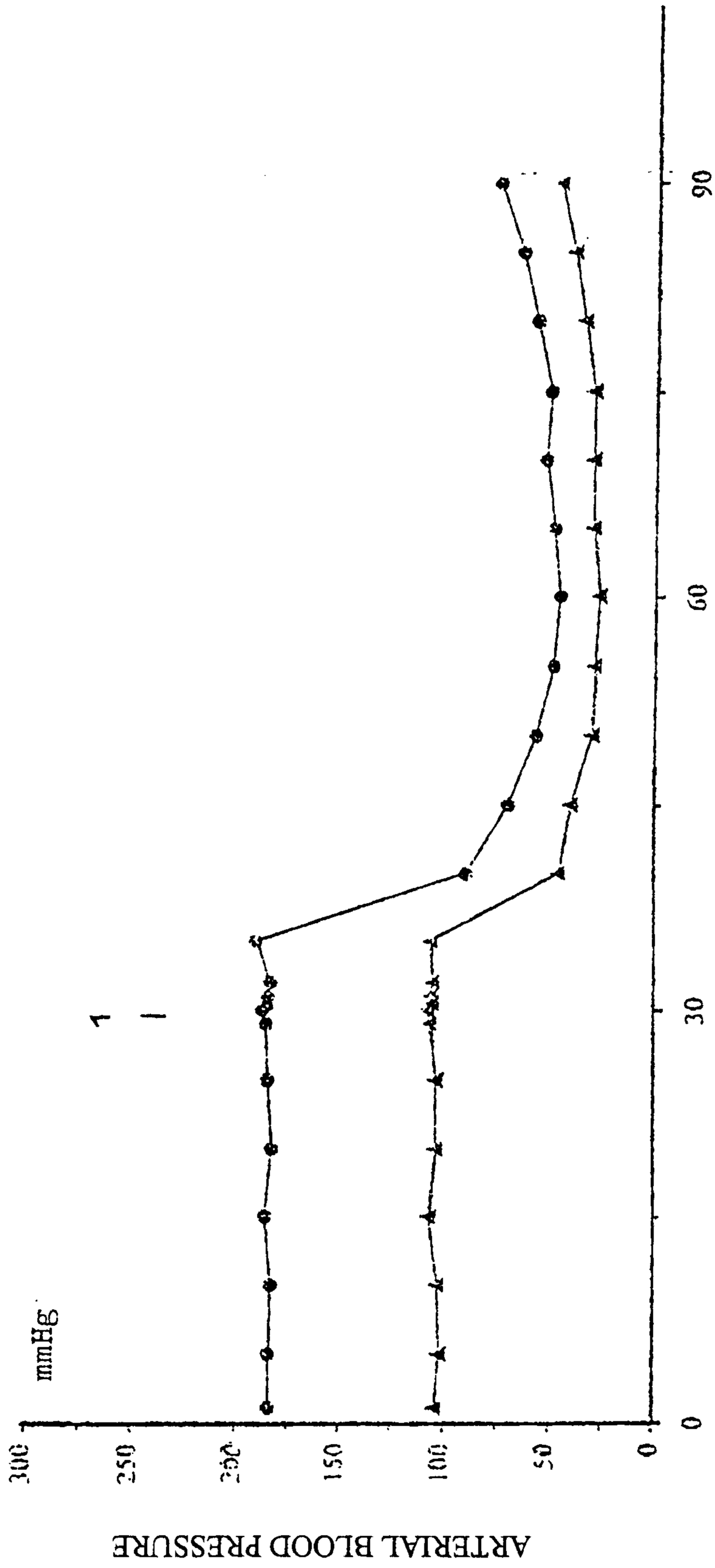

ARTERIAL BLOOD PRESSURE
mmHg
300
250
200
150
100
50
0
1
TIME
30
60
90
min.

FIGURE 3

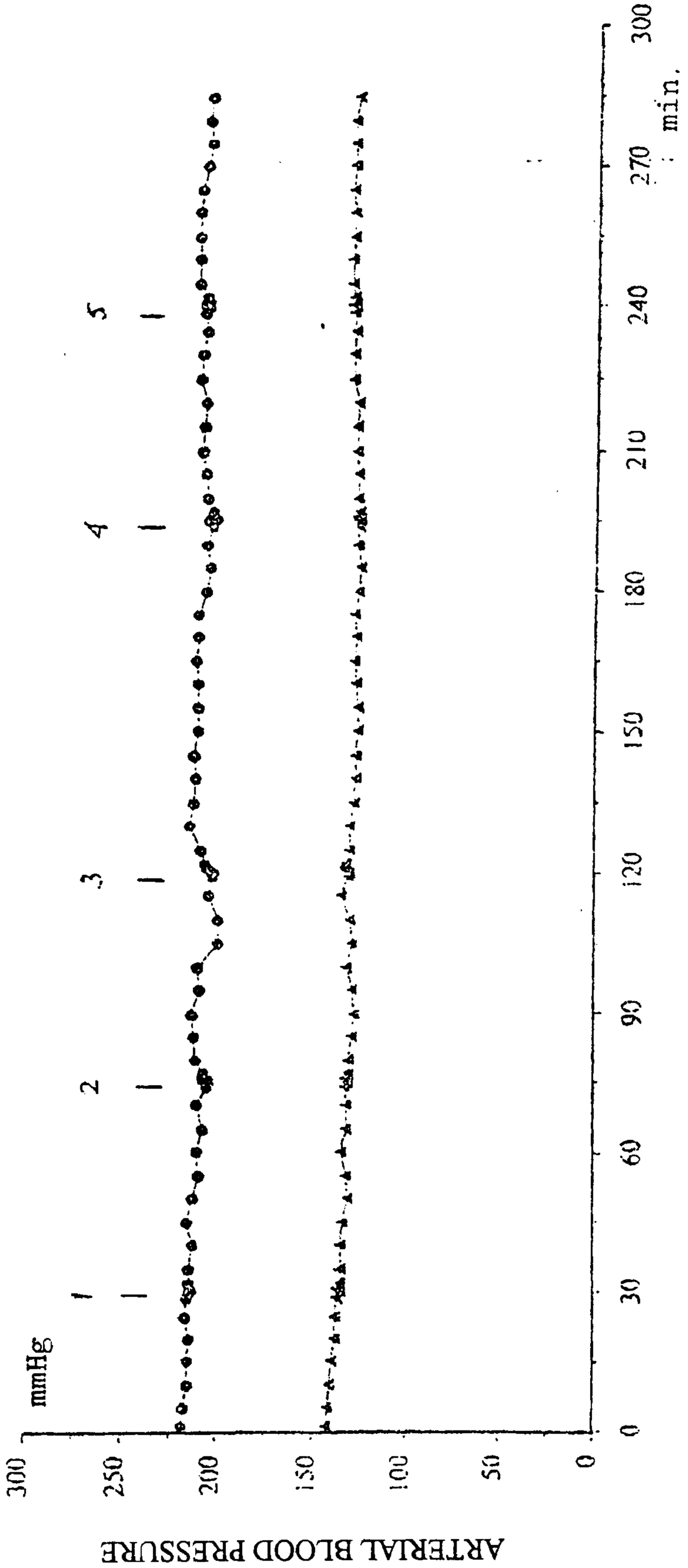

ARTERIAL BLOOD PRESSURE
mmHg
300
250
200
150
100
50
0
1
2
3
4
5
0
30
60
90
120
150
180
210
240
270
300
min.
TIME

FIGURE 4

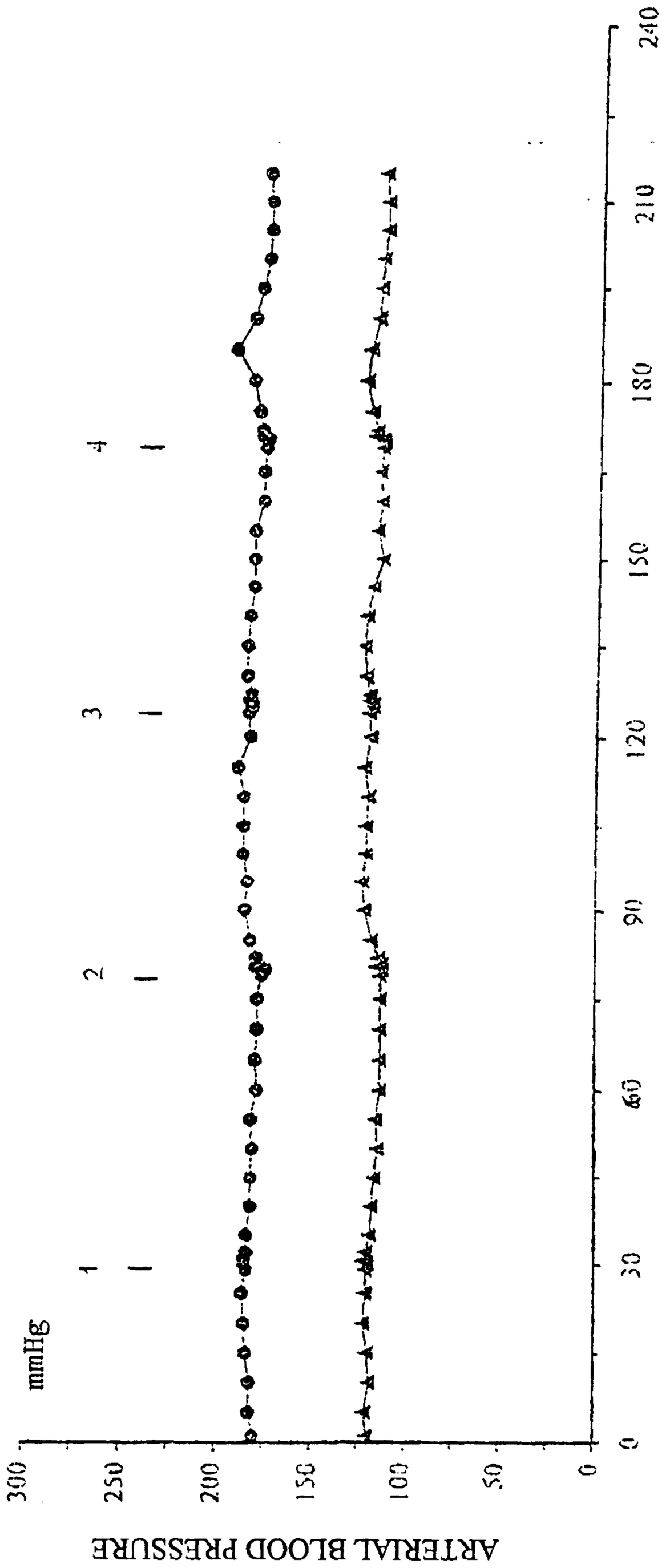

ARTERIAL BLOOD PRESSURE
mmHg
300
250
200
150
100
50
0
1
1
2
1
3
1
4
1
0
30
60
90
120
150
180
210
240
min.
TIME

FIGURE 5

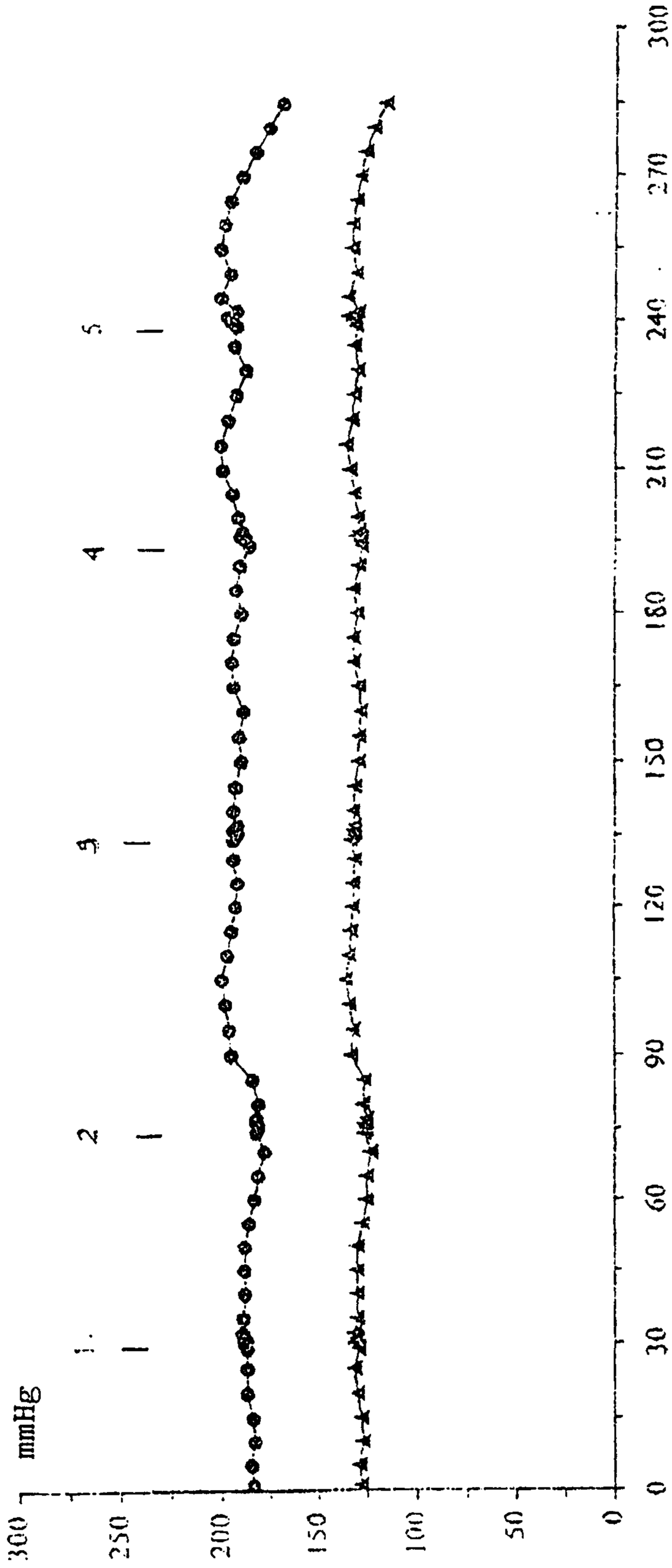

ARTERIAL BLOOD PRESSURE
mmHg
300
250
200
150
100
50
0
1
2
3
4
5
0
30
60
90
120
150
180
210
240
270
300
min.
TIME

FIGURE 6

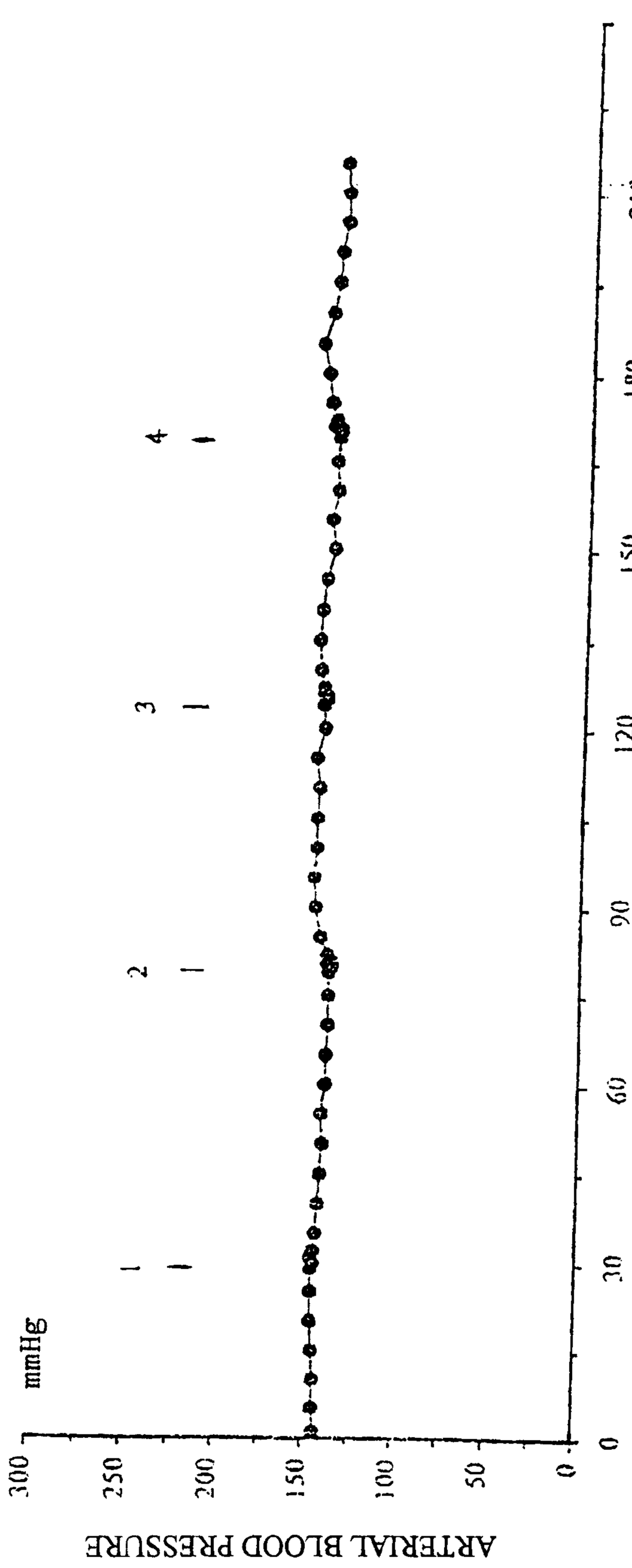
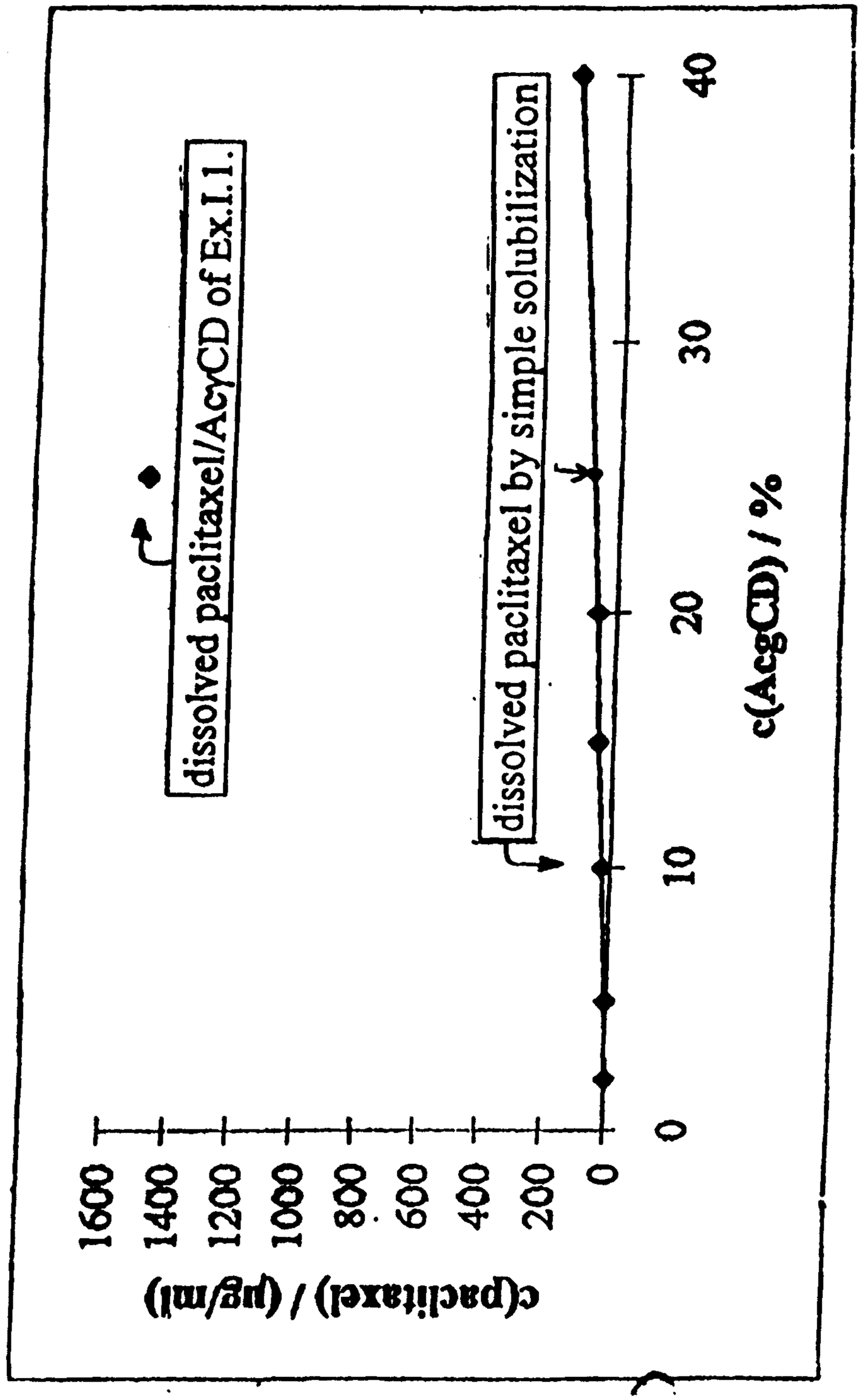

ARTERIAL BLOOD PRESSURE
mmHg
300
250
200
150
100
50
0
TIME
240 min.
210
180
150
120
90
60
30
0
1
2
1
3
1
4
1

FIGURE 7


dissolved paclitaxel/AcyCD of Ex.I.1.
dissolved paclitaxel by simple solubilization
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
c(paclitaxel) / (µg/ml)
c(AcgCD) / %