Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2096888 C 2004/04/27

(11)(21) 2 096 888

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(22) Date de dépôt/Filing Date: 1993/05/25

(41) Mise à la disp. pub./Open to Public Insp.: 1993/11/27

(45) Date de délivrance/Issue Date: 2004/04/27

(30) Priorités/Priorities: 1992/05/26 (P 42 17 355.8) DE;
1992/11/28 (P 42 40 103.8) DE

(51) Cl.Int.^{5}/Int.Cl.^{5} C12N 7/04, A61L 2/04, A61K 38/36

(72) Inventeurs/Inventors:
KEUPER, HERMAN, DE;
MATZMORR, WALTER, DE;
FREUDENBERG, WILFRIED, DE

(73) Propriétaire/Owner:
DADE BEHRING MARBURG GMBH, DE

(74) Agent: BERESKIN & PARR

(54) Titre : METHODE D'INACTIVATION DES VIRUS DANS LES PREPARATIONS CONTENANT DES PROTEINES
(54) Title: PROCESS FOR THE INACTIVATION OF VIRUSES IN PREPARATIONS OF PROTEINS

(57) Abrégé/Abstract:
The invention relates to a process for the inactivation of viruses in a solution of proteins by brief heating.

2096888

BEHRINGWERKE AKTIENGESELLSCHAFT

92/B 013 J - Ma 924
Dr. Ha/hg

Abstract of the disclosure

Process for the inactivation of viruses in preparations of proteins

The invention relates to a process for the inactivation of viruses in a solution of proteins by brief heating.

BEHRINGWERKE AKTIENGESELLSCHAFT

92/B 013J - Ma 924
Dr. Ha/hg

Process for the inactivation of viruses in preparations of proteins

The invention relates to a process for the inactivation of viruses in preparations of proteins. To do this, a solution of the preparation of a protein is briefly heated.

Proteins within the meaning of the invention are placental proteins, plasma proteins, proteins from cell cultures or from microbial fermentations. An example of a protein of this type is tissue thromboplastin.

The reagents employed for the Quick determination of the prothrombin time contain tissue thromboplastin as active constituent. The prothrombin time is an important screening test in the diagnosis of coagulation disorders.

In addition, the therapeutic use of tissue thromboplastin as FVIII bypassing agent has been suggested for the treatment of hemophiliacs with inhibitors. The virus safety of the preparation is an indispensable prerequisite for this use. However, virus safety is also required on use of tissue thromboplastin as diagnostic aid, having regard to the preservation of the health of the user.

At present, tissue thromboplastins for preparing prothrombin time reagents are normally obtained from brain or placenta of mammals. Contaminations by viruses such as HBV, HCV, HIV for preparations of human origin or by the agent causing BSE in the case of cattle cannot in principle be ruled out with preparations of this type. This means that a process for virus inactivation in tissue thromboplastin preparations has great importance.

To date, attempts at virus inactivation by established processes (detergents, hypochlorite, UV/gamma irradiation etc.) have failed because of the great sensitivity of the preparations. In particular, all attempts at pasteurization or dry heating have been unsuccessful.

It has emerged, surprisingly, that a brief heating, for example in an apparatus disclosed in Chem.-Ing.-Tech. 62 (1990), 486-487 (German Patent Application 39 05 066), has no adverse effect on the properties of tissue thromboplastin but, on the other hand, completely inactivates viruses. In the known processes for virus inactivation (pasteurization, "dry heating"), heating is customarily carried out for at least one minutes, but usually for several hours.

The invention relates to a process for the inactivation of viruses in a preparation of a protein from the group of placental proteins, plasma proteins or proteins prepared in cell culture or microbially, which comprises heating a solution of this preparation for a short time.

Heating is carried out with indirect heating in a heat exchanger. Suitable heat exchangers have any desired type of construction, such as plate exchangers or tubular exchangers.

A heat exchanger as disclosed in German Patent Application 39 05 066 is particularly suitable because, while the heat transfer coefficient is high, a short residence time and a lower wall temperature is possible.

This heat exchanger is a heat exchange module composed of stacked metal foils with spacers arranged between them, the metal foils being composed of metal cards which are provided with at least 2 openings on each of the opposite sides, the spacers being composed of fabric cards with

openings which are coincident with the metal cards so that the openings form tubular channels when the cards are stacked, the encircling rim of the fabric cards and an annular area of the fabric which encloses some of the openings being filled with sealing agent, there being alternation of the openings with and without sealing agent in a row of a fabric card and in the case of the tubular channels.

The heating temperature can be between +45°C and +95°C, but preferably between +65°C and 80°C.

In order that the fed-in liquid can be heated in the minimum time to the particular heating temperature and subsequently cooled down again in the same time, the apparatus in which heating is carried out is expediently equipped with a connector each for heating medium and cooling medium.

The constructional design of the heat exchangers ought to be such that the difference between the heating temperature for the solution and the temperature of the heating medium is a minimum, which results in minimum damage to the product because of the low wall temperature resulting therefrom.

The heating and/or cooling time should be less than 30 seconds, but preferably less than 5 seconds. The dwell time can be between 0.1 and 20 s, but preferably between 0.5 and 5 seconds.

The protein preparation can be, for example, a therapeutic agent containing tissue thromboplastin, or a diagnostic agent containing tissue thromboplastin, for example a prothrombin time reagent.

In the following example, the virus inactivation by brief heating is shown, without limiting the invention, by the

example of ᴿThromborel S, a prothrombin time reagent containing tissue thromboplastin from human placenta of Behringwerke AG. The success of the inactivation was examined. Complete virus inactivation was found at a heating temperature above 45°C, preferably at least 65°C. The diagnostic properties of the briefly heated reagent were compared with those of untreated ᴿThromborel S. No disadvantageous changes in these properties were observed at least up to a heating temperature of 75°C.

Example

Procedure for the brief heating

For this, two heat exchangers (W 1.1 and W 1.2, Fig. 1) were mounted in a common fixing device which essentially corresponds to commercially available ultrafiltration cassette holders. The heat exchangers had a modular construction and were separated from one another by a specially constructed spacer plate.

This made it possible to connect the heat exchangers in such a way that the fed-in liquid was heated to the particular heating temperature within 2 seconds and subsequently cooled down again in the same time. For this purpose, the apparatus was equipped with a connector each for heating medium and cooling medium. The dwell time at the said heating temperature was about 1.5 seconds.

Before the process started, the entire system on the product side was flushed with water and heated to the operating temperature. For this, the pump P1 (Fig. 1) transported heating medium through the system until the required heating temperature was reached. It was then changed from water to the solution to be heated, and in control tests the pump P2 was subsequently switched on to meter the virus suspension. Samples were taken before heating and after the experimentally determined minimum test time of 20 or 48 seconds.

Results of the virus inactivation (Table 1)

As a control, a solution of tissue thromboplastin was passed at room temperature through the system. The results show that a virus inactivation of more than 5 powers of ten was achieved in all tests on the coat-free and heat-resistant poliovirus. The inactivation was likewise complete in the case of the herpesvirus HSV-1.

The inactivation was examined for 2 different initial titers (about 3.5 and 5.0) and was complete in both case. By contrast, in the control experiment, virus inactivation was undetectable or only low.

Table 1

Virus inactivation after brief heating in ^{R}Thromborel S

Virus	Product throughput [l/h]	Virus dose [ml/h]	Titer of the virus stock [$\log_{10}$ $CCID_{50}$/ml]	Temperature [°C]	Virus titer [$\log_{10}$ $CCID_{50}$/ml] before heating	after heating	Virus inactivation factor [$\log_{10}$ $CCID_{50}$/ml]
HSV-1	50	480	6.1	25	3.8	3.5	0.3
Polio II	50	480	7.0	25	5.1	5.1	0
HSV-1	50	480	6.1	65	3.6	< 0	> 3.6
HSV-1	50	900	8.0	65	5.5	< 0	> 5.5
Polio II	50	480	7.0	65	5.1	< 0	> 5.1
HSV-1	50	480	6.1	70	3.6	< 0	> 3.6
HSV-1	50	900	8.0	70	5.1	< 0	> 5.1
Polio II	50	480	7.0	70	5.0	< 0	> 5.0
HSV-1	50	480	6.1	75	3.4	< 0	> 3.4
Polio II	50	480	7.0	75	5.1	< 0	> 5.1

Comparison of briefly heated with untreated tissue thromboplastin

[R]Thromborel S was briefly heated at various temperatures in the above system without metering in virus suspension. Untreated material served as control. All the preparations were freeze-dried and reconstituted before testing in the same volume of distilled water. The results which are presented below were obtained with a representative batch.

To construct the reference plots (Fig. 2), standard human plasma from Behringwerke AG was employed undiluted and diluted with isotonic sodium chloride solution as sample. Sample (100 μl) and reagent (200 μl) were mixed and the clotting time was measured in a Schnitger & Gross coagulometer. The reference plots for heated and untreated [R]Thromborel S are essentially identical.

The sensitivity of prothrombin time reagents is expressed by ISI (international sensitivity index). The ISI values for heated and untreated [R]Thromborel S, determined on plasma from healthy subjects and subjects undergoing oral anticoagulation, compared with a reference thromboplastin are indistinguishable (Tab. 2).

Table 2

	International Sensitivity Index	
	Determination 1	Determination 2
Control	1.09	1.09
65°C	1.12	1.09
75°C	1.10	1.12
80°C	1.10	1.10

To characterize the sensitivity of heated and untreated ®Thromborel S to the coagulation factors II, V, VII and X, standard human plasma was mixed with the appropriate coagulation factor-deficient plasma from Behringwerke AG to adjust the activities of the relevant factor to between 5 and 100% of normal. The clotting times of the mixtures (100 µl) were determined after additions of 200 µl of heated or untreated ®Thromborel S in a Schnitger & Gross coagulometer. The clotting time is plotted against the content of factor II and F VII, respectively, as a percentage of normal in Fig. 3 and 4; this revealed that the sensitivity to the tested factors was as good as for the control. The sensitivities for factor V and factor X (not shown) were indistinguishable within the accuracy of measurement.

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

1. A process for the inactivation of viruses in a preparation of a protein selected from the group consisting of placental proteins, plasma proteins, proteins prepared in cell culture and proteins prepared by microbes, which comprises indirectly heating a solution of the protein preparation in a heat exchanger at a temperature between +65°C and +80°C with a heating and cooling time of 2 seconds and a dwell time between 0.1 and 20 seconds.

2. The process as claimed in claim 1, wherein a solution of tissue thromboplastin is heated.

3. The process as claimed in claim 1 or 2, wherein the dwell time is between 0.5 and 5 seconds.

4. The process as claimed in any one of claims 1-3, wherein heating is carried out in a heat exchange module composed of stacked metal foils with spacers arranged between them, the metal foils being composed of metal cards which are provided with a least 2 openings on each of the opposite sides, the spacers being composed of fabric cards with openings which are coincident with the metal cards so that the openings form tubular channels when the metal cards and the fabric cards are stacked, the encircling rim of the fabric cards and an annular area of the fabric which encloses some of the openings being filled with sealing agent, there being alternation of the opening with and without sealing agent in a row of a fabric card and in the case of the tubular channels.

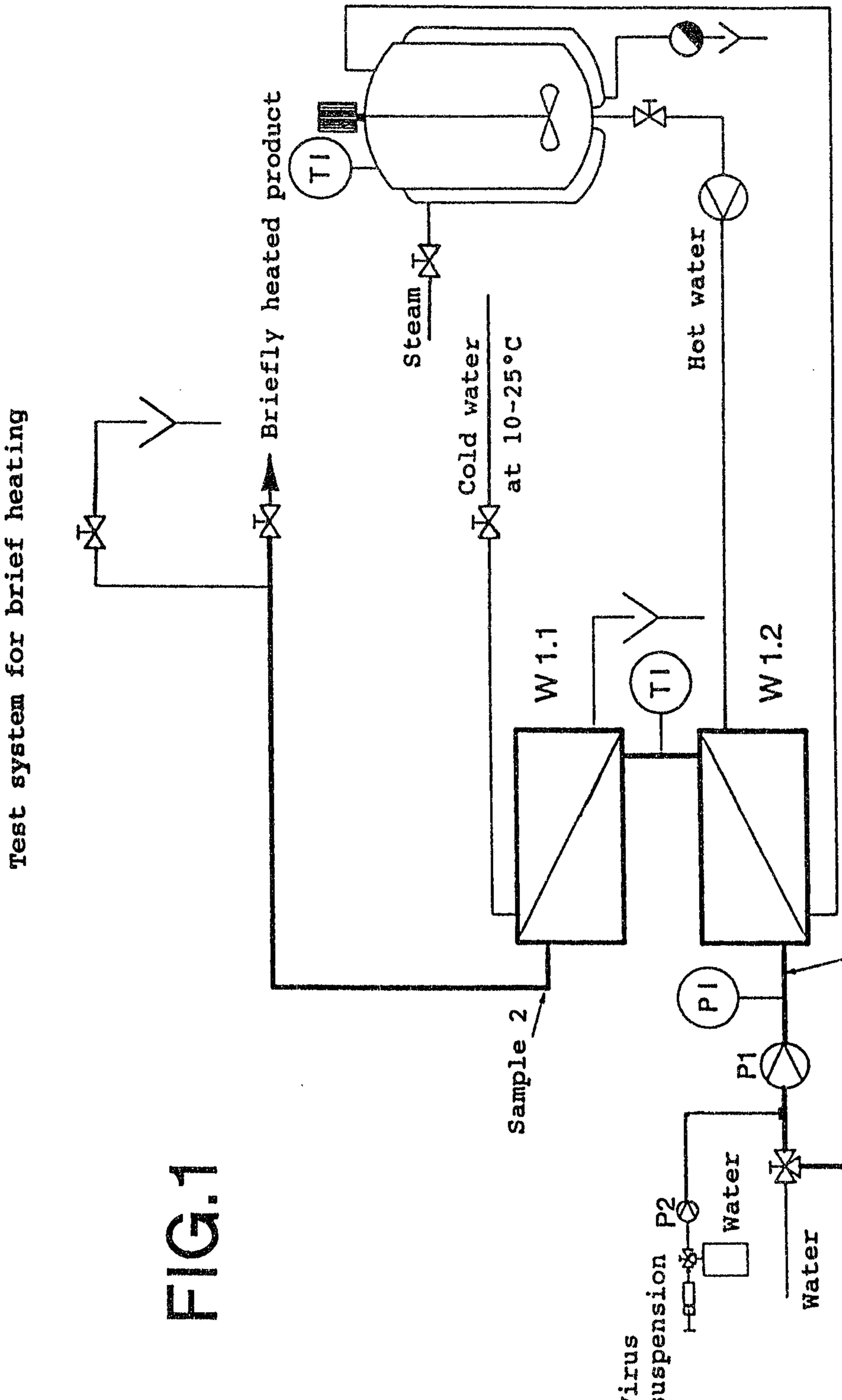
Test system for brief heating
Briefly heated product
Sample 2
Steam
Cold water
at 10-25°C
Hot water
W 1.1
TI
W 1.2
PI
Sample 1
P1
Protein
solution
P2
Water
Water
Virus
suspension

FIG.1

Brief heating of Thromborel S
Reference plots
Batch No. 0291

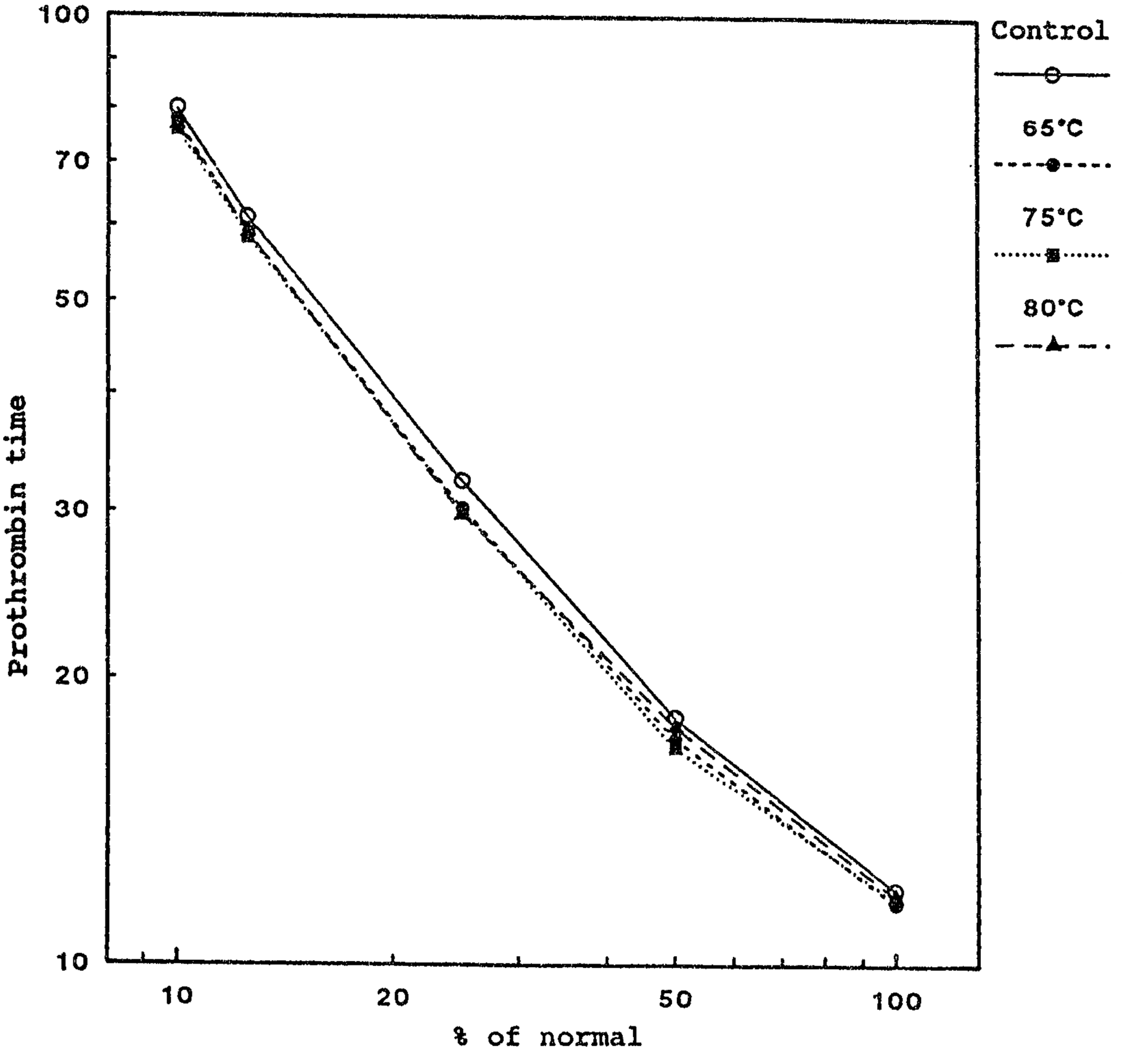

FIG.2

Brief heating of Thromborel S
FII Sensitivity
Batch No. 0291

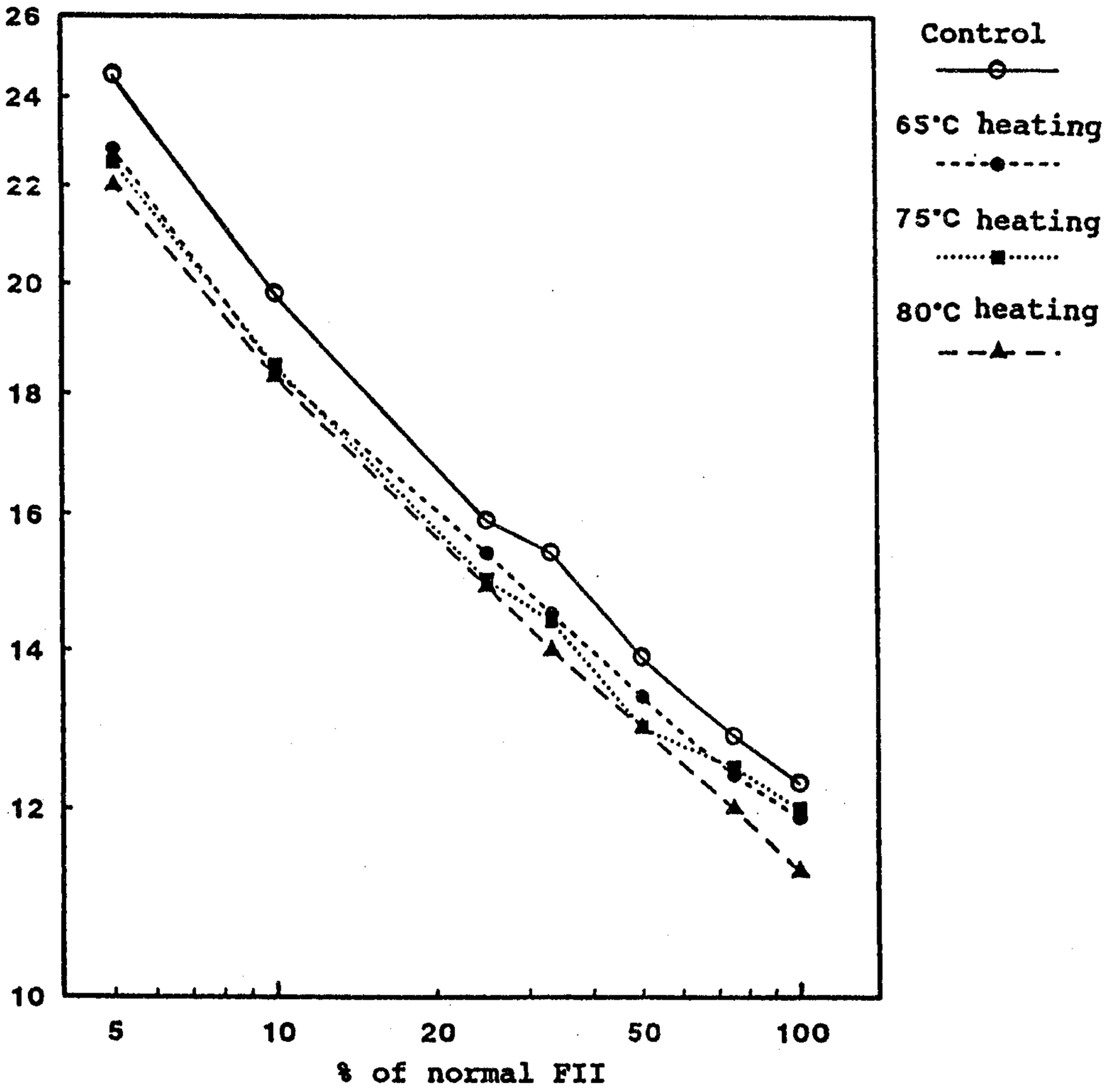

FIG.3

Brief heating of Thromborel S
FVII Sensitivity
Batch No. 0291

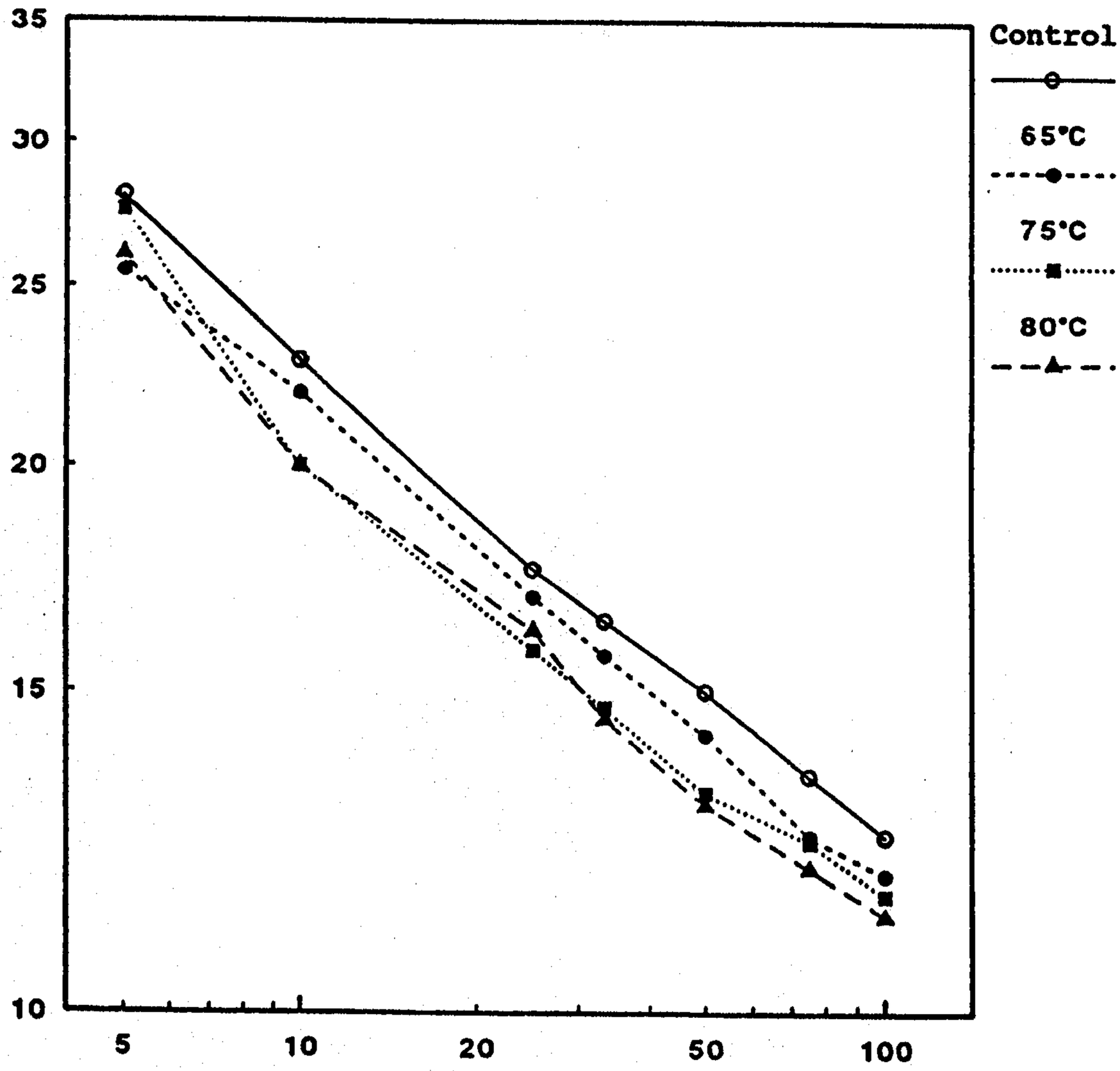

FIG.4