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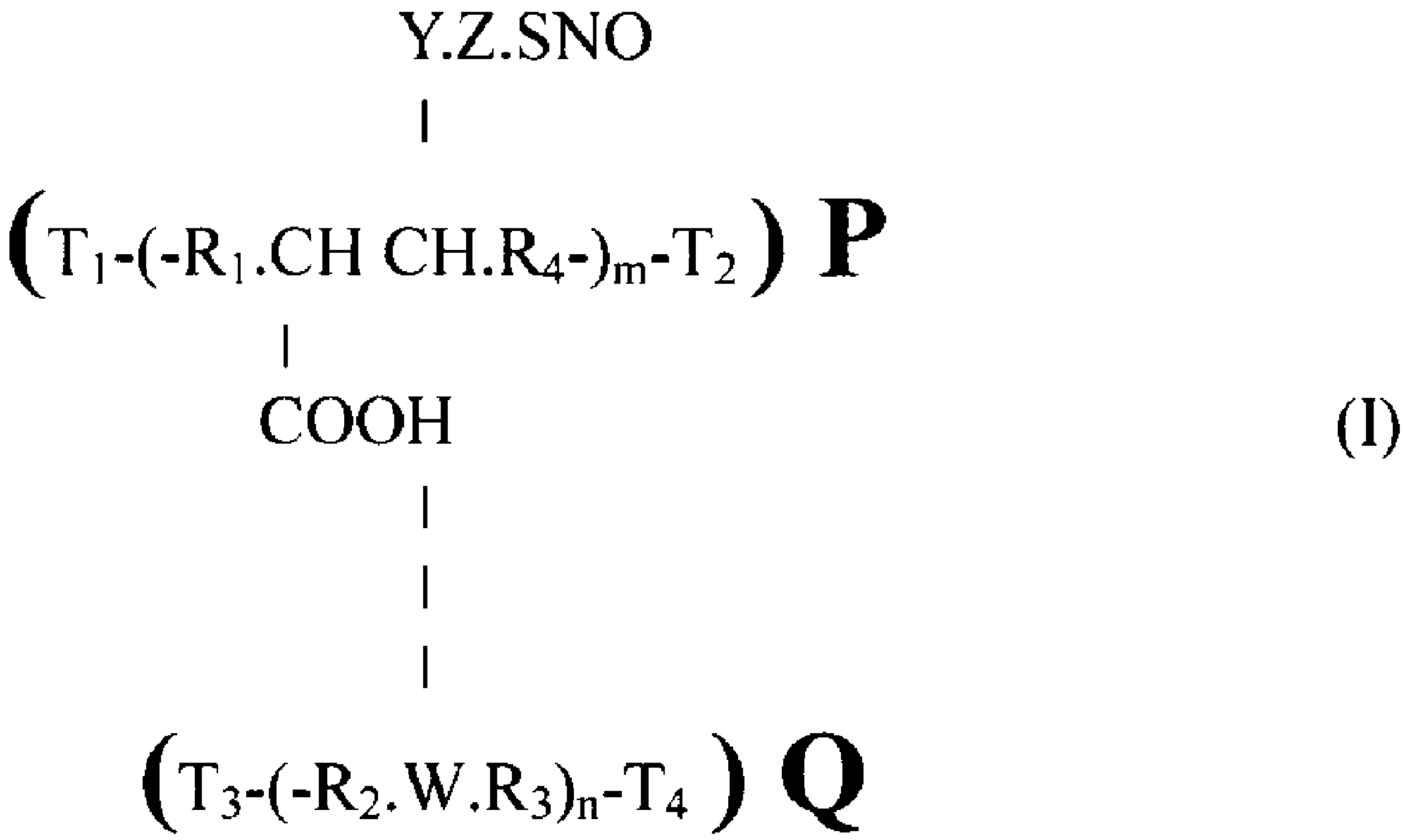
(54) Titre : COMPLEXES POLYMERES SUPRA-MACROMOLECULAIRES PERMETTANT DE REGULER LA LIBERATION D'OXYDE NITRIQUE DANS DES DISPOSITIFS DE CICATRISATION DE BLESSURES

(54) Title: SUPRAMACROMOLECULAR POLYMER COMPLEXES PROVIDING CONTROLLED NITRIC OXIDE RELEASE FOR HEALING WOUNDS



(I)

(57) Abrégé/Abstract:
A bio-adhesive supramacromolecular complex of the general formula (I): (see formula I) wherein R_1 is an alkane unsubstituted or substituted with alkoxy groups; R_2 is a lower alkane; R_3 and R_4 are long chain, optionally substituted, alkanes; W is a hydrogen-

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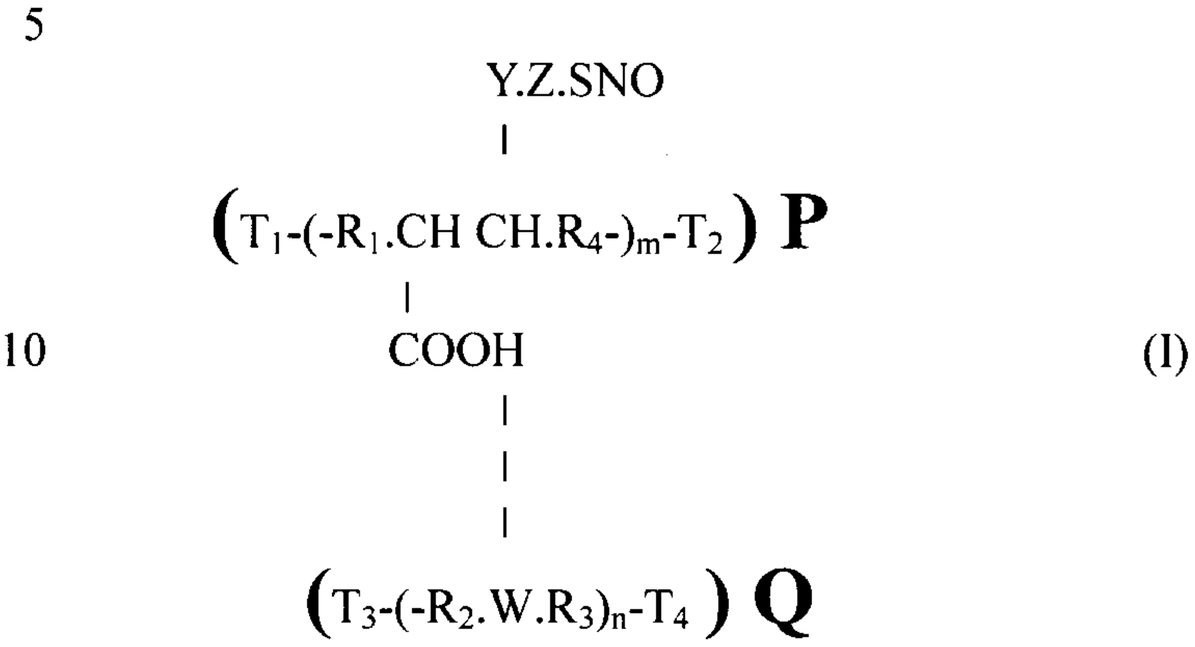
(57) Abrégé(suite)/Abstract(continued):
bond accepting functional group-containing entity; Y is a carboxylic acid ester or amide; Z is a linking group; T_1, T_2, T_3 and T_4 are terminal groups; m and n are integers selected from at least 25; and wherein P has a molecular weight of about 1×10^3 to 1×10^6 and Q has a molecular weight of about 1×10^3 to 1×10^7. The complex provides controlled nitric oxide release over a longer period of time than prior art compounds in the healing of wounds and infections. Novel compositions, methods of preparation, skin coverings containing and medical use of the complexes are described.

ABSTRACT OF THE DISCLOSURE

A bio-adhesive supramacromolecular complex of the general formula (I):



(I)

wherein R_1 is an alkane unsubstituted or substituted with alkoxy groups;

R_2 is a lower alkane;

R_3 and R_4 are long chain, optionally substituted, alkanes;

W is a hydrogen-bond accepting functional group-containing entity;

Y is a carboxylic acid ester or amide;

Z is a linking group;

T_1, T_2, T_3 and T_4 are terminal groups;

m and n are integers selected from at least 25; and wherein P has a molecular weight of about 1×10^3 to 1×10^6 and Q has a molecular weight of about 1×10^3 to 1×10^7. The complex provides controlled nitric oxide release over a longer period of time than prior art compounds in the healing of wounds and infections. Novel compositions, methods of preparation, skin coverings containing and medical use of the complexes are described.

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SUPRAMACROMOLECULAR POLYMER COMPLEXES PROVIDING CONTROLLED NITRIC OXIDE RELEASE FOR HEALING WOUNDS

FIELD OF THE INVENTION

This invention relates to supramacromolecular nitric oxide releasing polymer complexes; compositions and impregnated and coated articles comprising said complexes; methods of making said complexes; and methods of using said complexes, compositions and articles in the treatment of healing wounds, particularly cuboidal ulceration caused by diabetes.

BACKGROUND OF THE INVENTION

Nitric oxide is known to play a key physiological role in the promotion of endothelial cell proliferation, protection of endothelial cells from apoptosis, and inhibition of inflammatory cell adhesion [1]. As a result, topical exposure of nitric oxide gas has been shown to be potentially beneficial in promoting the healing of chronic non-healing wounds, such as diabetic ulcers [2, 3]. However, the short half life and intrinsic instability of this small gaseous molecule have prevented it from being incorporated into pharmaceutical formulations and drug delivery systems.

Various nitric oxide precursors or donors, such as diazeniumdiolates and nitrosothiols doped or grafted polymers have been synthesized to overcome this drawback [4]. However, diamine-based and polyethylenimine-based diazeniumdiolates released into aqueous medium have been shown to form measurable levels of nitrosamines, a known class of carcinogens [5]. On the other hand, S-nitrosoglutathione (GSNO), a nitrosothiol, has attracted significant attention due to its endogenous occurrence and its ease of synthesis through a spontaneous reaction between glutathione and sodium nitrite at room temperature [6]. GSNO has been physically incorporated into polymer carriers such as PVA, PVP and Pluronic hydrogels [7, 8] or covalently attached to polymers such as BSA and PEG [9], in an attempt to improve biocompatibility and prolong the NO release duration. However, these reported hydrophilic systems lack the desired stability as the S-NO bond is both thermally and photolytically

labile, and susceptible to hemolytic cleavage leading to the spontaneous release of NO and its rapid inactivation. As a result, the nitric oxide release duration from compounds of the prior art cannot be maintained for any extended period, which is, generally, not more than several hours.

Prior art methods of either physically mixing GSNO in a polymer [7, 8] to form an admixture or to mix a NO precursor with an activator at the time of application to generate GSNO in situ, as described in e.g. WO2006/095193, to regulate the NO release, do not address the issue of short half-life of GSNO, because once GSNO is formed or released, it is still susceptible to degradation due to heat, moisture and light. In fact, in most of these prior art approaches, the release of NO or GSNO, is usually very rapid and lasts no more than several hours.

There is, therefore, a need for a nitric oxide carrier that provides a durable release of nitric oxide for use in the healing of wounds.

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[4] Frost MC, Reynolds MM, Meyerhoff ME. Polymers incorporating nitric oxide releasing/generating substances to improve biocompatibility of blood-contacting medical devices. Biomaterials 2005;26:1685-693.

[5] Mowery KA, Sochoenfisch MH, Saavedra JE, Keefer LK, Meyerhoff ME. Preparation and characterization of hydrophobic polymeric films that are thromboresistant via nitric oxide release. Biomaterials 2000;21:9-21.

[6] Butler AR, Rhodes P, Chemistry, analysis, and biological roles of S-nitrosothiols, Anal Biochem 1997; 249:1–9.

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[8] Seabra AB, Fitzpatrick A, Paul J, de Oliveira MG, Weller R. Topically applied S-nitrosothiol-containing hydrogels as experimental and pharmacological nitric oxide donors in human skin. Brit J Dermatol 2004;151:977–83.

[9] Katsumi H, Nishikawa M, Yamashita F, Hashida M. Development of polyethylene glycol-conjugated poly-S-nitrosated serum albumin, a novel S-nitrosothiol for prolonged delivery of nitric oxide in the blood circulation in vivo. J Pharmcol Exp Therap 2005;314:1117–24.

SUMMARY OF THE INVENTION

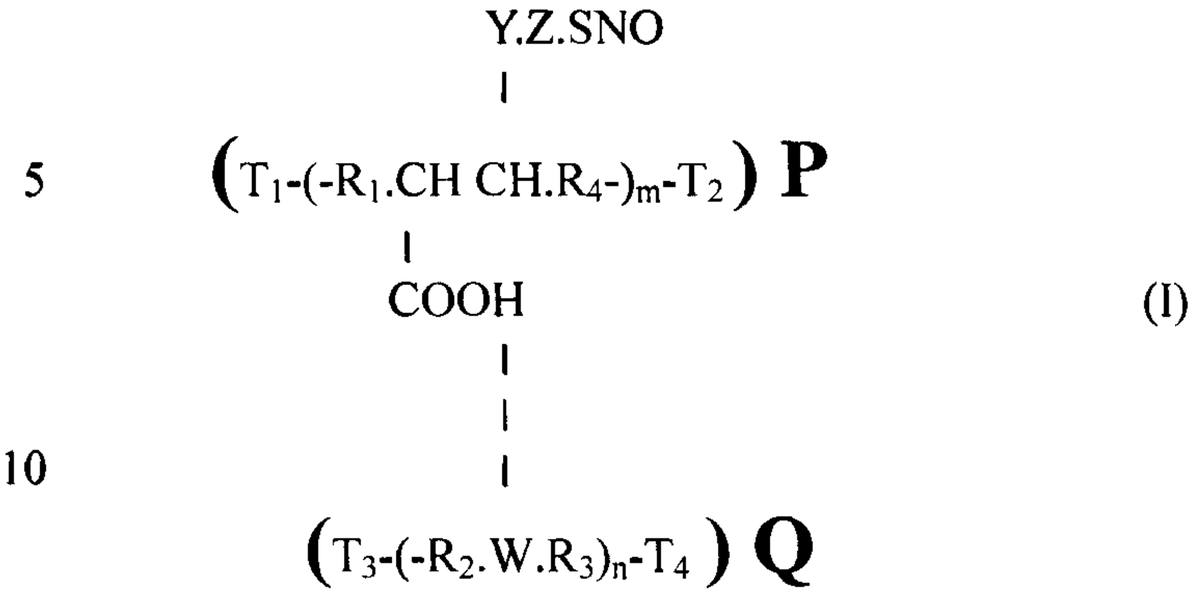
It is an object of the present invention to provide a nitric oxide carrier that provides a simple, stable and biocompatible means for generating a durable release of nitric oxide in the healing of wounds.

It is a further object to provide a method of making said nitric oxide carrier.

It is a further object to provide said nitric oxide carrier in the form of several physical forms, such as a powder or coating.

The invention provides a bioadhesive supramacromolecular complex comprising the product of a nitric oxide donor covalently linked to a hydrophobic bioadhesive polymeric polyanhydride, intermolecularly hydrogen bonded to a polymeric.

Accordingly, in one aspect the invention provides a bio-adhesive supramacromolecular complex of the general formula (I):



wherein R_1 is an alkane unsubstituted or substituted with alkoxy groups;

R_2 is a lower alkane;

R_3 and R_4 are long chain, optionally substituted, alkanes;

W is a hydrogen-bond accepting functional group-containing entity;

Y is a carboxylic acid ester or amide;

Z is a linking group;

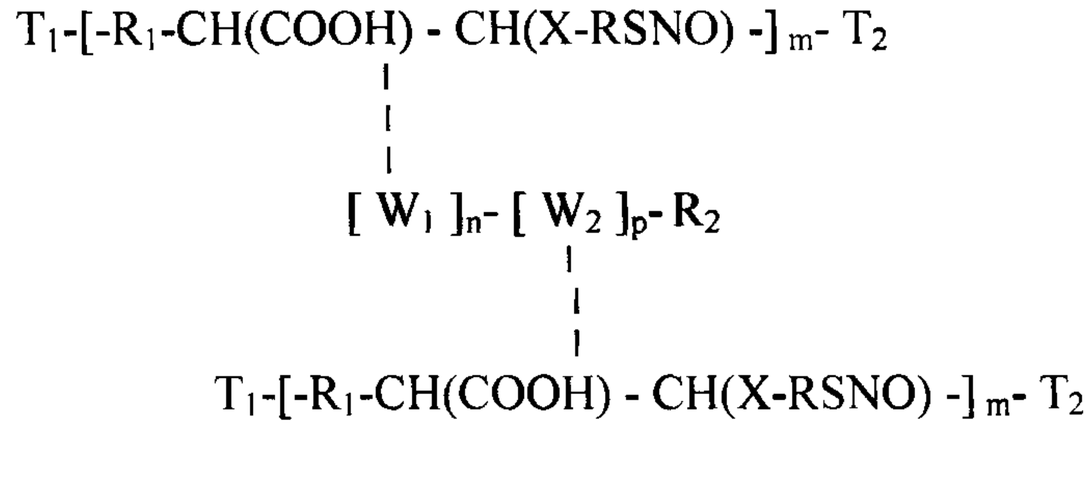
T_1, T_2, T_3 and T_4 are terminal groups;

m and n are integers selected from at least 25; and wherein P has a molecular weight of about 1×10^3 to 1×10^6 and Q has a molecular weight of about 1×10^3 to 1×10^7.

Preferred P values range from about 4×10^3 to 2×10^6, and preferred Q values range from about 3×10^3 to 7×10^6.

The supramacromolecular complex is, preferably, wherein R_1 is a maleic acid copolymer, and more preferably, wherein the maleic acid copolymer is selected from the group consisting of poly(methyl vinyl ether-co-maleic acid) poly(vinylpyrrolidone-co-dimetyl maleic acid), poly(ethylene-co-maleic acid), poly(isobutylene-co-maleic acid), poly(styrene-co-maleic acid), poly(ethylene-co-ethyl acrylate-co-maleic aci), poly(maleic acid-co-octadecene), polyethylene-graft-maleic acid, polypropylene-graft-maleic acid, and polyisoprene-graft-maleic acid.

In a further aspect the invention provides a bio-adhesive supramacromolecular complex of the general formula:



wherein R_1 is an alkyl vinyl ether ($C_1 - C_5$), ethylene, propylene, isobutylene, butadiene, 1-octadecene, styrene, maleic acid, or maleic anhydride unit;

W_1 and W_2 are hydrogen-bond accepting functional group-containing entities selected from vinylpyrrolidone, ethylene oxide or propylene oxide, and vinyl acetate;

R_2 is H, a fatty acid ester, or fatty alcohol;

X is a carboxylic acid ester or amide linkage;

RSNO is a S-nitrosothiol of cysteine, γ-Glu-Cys, α-Glu-Cys, glutathione, homoglutathione, hydroxymethyl-glutathione, γ-Glu-Cys-Glu, α-Glu-Cys-Gly, α-Glu-Cys-β-Ala, α-Glu-Cys-Ser, α-Glu-Cys-Glu, other glutathione analog containing –SH and $–NH_2$ and/or –OH functional groups, or one of the following peptides: $(\gamma\text{-Glu-Cys})_q$, $(\gamma\text{-Glu-Cys})_q$-Gly, $(\gamma\text{-Glu-Cys})_q$-β-Ala, $(\gamma\text{-Glu-Cys})_q$-Ser, $(\gamma\text{-Glu-Cys})_q$-Glu, $(\alpha\text{-Glu-Cys})_q$, $(\alpha\text{-Glu-Cys})_q$-Gly, $(\alpha\text{-Glu-Cys})_q$-β-Ala, $(\alpha\text{-Glu-Cys})_q$-Ser, and $(\alpha\text{-Glu-Cys})_q$-Glu, where q=2-7;

T_1 and T_2 are terminal groups;

n, m, and p are integers greater than 25.

The supramacromolecular complex is, preferably, wherein T_1-[-R_1-CH(COOH) - CH(X-RSNO) -] $_m$- T_2 is a reaction adduct of RSNO and a maleic anhydride polymer or copolymer, wherein the maleic anhydride polymer or copolymer is selected from the group consisting of poly(methyl vinyl ether-alt-maleic anhydride), poly(maleic acid-co-maleic anhydride), poly(maleic anhydride), poly(vinylpyrrolidone-co-dimethyl maleic anhydride), poly(vinylacetate-co-maleic anhydride), poly(ethylene-alt-maleic anhydride), alt-maleic

poly(isobutylene-anhydride), poly(styrene-alt-maleic anhydride), poly(ethylene-co-ethyl acrylate-co-maleic anhydride), and poly(maleic anhydride-alt-1-octadecene).

The nitric oxide donor RSNO is, preferably, selected from the group consisting of S-nitrosothiols of cysteine, γ-Glu-Cys, α-Glu-Cys, glutathione, homoglutathione, hydroxymethyl-glutathione, γ-Glu-Cys-Glu, α-Glu-Cys-Gly, α-Glu-Cys-β-Ala, α-Glu-Cys-Ser, α-Glu-Cys-Glu, other glutathione analog containing –SH and $–NH_2$ and/or –OH functional groups, or one of the following peptides: $(\gamma\text{-Glu-Cys})_n$, $(\gamma\text{-Glu-Cys})_n$-Gly, $(\gamma\text{-Glu-Cys})_n$-β-Ala, $(\gamma\text{-Glu-Cys})_n$-Ser, $(\gamma\text{-Glu-Cys})_n$-Glu, $(\alpha\text{-Glu-Cys})_n$, $(\alpha\text{-Glu-Cys})_n$-Gly, $(\alpha\text{-Glu-Cys})_n$-β-Ala, $(\alpha\text{-Glu-Cys})_n$-Ser, and $(\alpha\text{-Glu-Cys})_n$-Glu, where n=2-7.

The $T_3(-R_2W.)_n-(R_3)_p-(-R_2W)_n-T_3$ and the $[\ W_1\ ]_n-[\ W_2\ ]_p-R_2$ hydrogen bond accepting polymer is, preferably, selected from the group consisting of poly(vinyl pyrolidone), polyethylene glycol, poly(ethylene oxide), poly(vinyl pyrrolidone-co-vinyl acetate), polyethylene oxide-polypropylene oxide block copolymers (Pluronics or Polaxomers), polyethylene glycol fatty alcohol esters, and polyethylene glycol fatty acids esters, and more preferably, poly(vinyl pyrolidone).

Preferably, Y.Z.SNO is an amido-S-nitrosoglutathione.

In a further aspect, the invention provides a method of making a bio-adhesive, supramacromolecular nitric oxide generatable polymer complex, said method comprising

(i) covalently linking a S-nitroso compound having an amino linking group with a bio-adhesive, hydrophobic polyanhydride compound to form a nitric oxide donor polymeric carrier; and

(ii) mixing said carrier with an hydrophilic intermolecular hydrogen bond-acceptable polymer to produce said supramacromolecular nitric oxide generatable complex.

Preferred nitric oxide donor RSNO is selected from the group consisting of S-nitrosothiols of cysteine, γ-Glu-Cys, α-Glu-Cys, glutathione, homoglutathione, hydroxymethyl-glutathione, γ-Glu-Cys-Glu, α-Glu-Cys-Gly, α-Glu-Cys-β-Ala, α-Glu-Cys-Ser, α-Glu-Cys-Glu, other glutathione analog containing –SH and $–NH_2$ and/or –OH functional groups, or one of the following peptides: $(\gamma\text{-Glu-Cys})_n$, $(\gamma\text{-Glu-Cys})_n$-Gly, $(\gamma\text{-Glu-Cys})_n$-β-Ala, $(\gamma\text{-Glu-Cys})_n$-Ser, $(\gamma\text{-Glu-Cys})_n$-Glu, $(\alpha\text{-Glu-Cys})_n$, $(\alpha\text{-Glu-Cys})_n$-Gly, $(\alpha\text{-Glu-}$

Cys)$_n$-β-Ala, (α-Glu-Cys)$_n$-Ser, and (α-Glu-Cys)$_n$-Glu, where n=2-7. Most preferably, the S-nitrosothiol compound is S-nitrosoglutathione.

Preferred polyanhydride compounds are maleic anhydride polymer or copolymers with molecular weight (Mw) ranging from about 5,000 to 2,000,000, wherein the maleic anhydride polymer or copolymer, for example, is preferably selected from the group consisting of poly(methyl vinyl ether-*alt*-maleic anhydride), poly(maleic acid-co-maleic anhydride), poly(maleic anhydride), poly(vinylpyrrolidone-co-dimethyl maleic anhydride), poly(vinylacetate-co-maleic anhydride), poly(ethylene-*alt*-maleic anhydride), poly(isobutylene-alt-maleic anhydride), poly(styrene-*alt*-maleic anhydride), poly(ethylene-co-ethyl acrylate-co-maleic anhydride), and poly(maleic anhydride-*alt*-1-octadecene). Most preferably, the polyanhydride compound is poly(methyl vinyl ether-*alt*-maleic anhydride).

The hydrogen bond accepting polymer is, preferably, selected from the group, with molecular weight (Mw) from about 5,000 to 7,000,000, consisting of poly(vinyl pyrrolidone), polyethylene glycol, poly(ethylene oxide), poly(vinyl pyrrolidone-co-vinyl acetate), polyethylene oxide-polypropylene oxide block copolymers (Pluronics or Polaxomers), polyethylene glycol fatty alcohol esters, and polyethylene glycol fatty acids esters, most preferably a method as claimed in claim 17 wherein said hydrogen bond acceptable polymer is poly(vinyl pyrrolidone).

The resulting supramacromolecular nitric oxide generatable polymer complex preferably contains a polyanhydride compound and a hydrogen bond accepting polymer in relative weight proportions ranging from 1:9 to 9:1, more preferably, 2:5 to 5:2, and most preferably 1:2 to 2:1.

The total loading of the nitric oxide donor RSNO in the resulting supramacromolecular nitric oxide generatable polymer complex is preferably in the range of 1 to 50 wt%, more preferably 10 to 40%, and most preferably 20 to 30%.

The invention, in a further aspect, provides a bio-adhesive, supramacromolecular nitric oxide generatable complex when made by a method as hereinabove defined.

In a yet further aspect, the invention provides a pharmaceutical composition comprising an effective wound healing amount of said supramacromolecular complex, as hereinabove defined, and a physiological acceptable carrier.

In a still yet further aspect, the invention provides a supramacromolecular complex, as hereinabove defined, in the physical form of a powder, spun fiber, or coating on a surface of a substrate, for example, a catheter or stent.

Thus, the present invention is directed to a novel nitric oxide-releasing polymer complex, which, in powder form, can serve as wound dressing and be incorporated into transdermal patches, bandages, sutures, and the like. It can also take the form of a coating by applying the polymer complex, prior to solidifying, to blood contacting surfaces on a medical device. This supramacromolecular complex produces a therapeutic amount of nitric oxide in a sustained and controlled manner and delivers it to the diseased tissues, such as those in chronic, poorly-healed wounds.

Thus, in a further aspect, the invention provides a skin covering for application to the skin, the covering incorporating an effective wound healing amount of a supramacromolecular complex, as hereinabove defined. The skin covering may be a bandage or wound dressing.

In a further aspect, the invention provides a method of enhancing the healing of a skin wound or infection, said method comprising applying an effective wound or infection healing amount of a bio-adhesive supramacromolecular complex or pharmaceutically acceptable composition thereof, as hereinabove defined, to said wound.

In a yet further aspect, the invention provides use of a bio-adhesive supramacromolecular complex or pharmaceutically acceptable composition thereof, as hereinabove defined, for enhancing the healing of a skin wound or infection.

Thus, the present invention comprises three essential key elements, namely, (1) a polymeric carrier which is hydrophobic, biocompatible, bioerodible and contains anhydride functional groups], for example, such as poly(methyl vinyl ether-*alt*-maleic anhydride) [PVMMA], (2) a nitric oxide donor such as S-nitrosoglutathione (GSNO) that can be covalently attached under mild conditions to the anhydride groups on the macromolecular backbone or side chain of the above polymeric carrier, for example, such as poly(vinyl pyrrolidone) [PVP]; and (3) a second polymer, which forms strong physical intermolecular complexes with the first polymeric carrier.

Thus, the field of the invention relates to devices and methods for treating wounds and infections, and more specifically, the treatment of wounds and infections with prolonged local release of nitric oxide. The complexes of the present invention can be made into

powders and incorporated in the bandage or wound dressing to facilitate wound healing. Additionally, it can be deployed as an ingredient of inhalation formulation to decrease pulmonary hypertension or applied to the treatment of circulation disorders.

Prolonged nitric oxide release from the bio-adhesive supramacromolecular complex over a period of at least about seven days provides efficacious treatment of wounds and infections. Without being bound by theory, we believe that the efficacy is due to the presence of the hydrogen bond-accepting functional group e.g. PVP, being hydrogen bonded through the carboxylic acid group of the bio-adhesive hydropholic polymer, e.g. PVMMA, which slows down the rate of formation of di-sulfide bonds and release of nitric oxide from sterically hindered GSNO embedded in the PVMMA hydrophobic mix.

BRIEF DESCRIPTION OF THE DRAWINGS

In order that the invention may be better understood, preferred embodiments will not be described, by way of example only, with reference to the drawings, wherein

Fig. 1 shows FTIR spectra of pure GSNO, pure PVMMA and GSNO-conjugated PVMMA films;

Fig. 2 shows FTIR spectra of pure PVMMA, PVP and PVM/MA/PVP complex;

Fig. 3 is a graph showing the in vitro release behaviour of NO from GSNO-PVMMA conjugate at several temperatures;

Fig. 4 is a graph showing the in vitro release behaviour of NO from GSNO-PVMMA/PVP supramacromolecular complex according to the invention at various PVMMA/PVP weight ratios;

Fig. 5 is a graph showing the in vitro release behaviour of NO from GSNO-PVMMA/PVP supramacromolecular complex according to the invention at several temperatures; and

Figs. 6A and 6B are graphs showing the in vitro release behaviour of NO from GSNO-PVMMA/PVP supramacromolecular complexes, according to the invention of different molecular weights of PVMMA (Fig. 6A) and PVP (Fig. 6B), respectively.

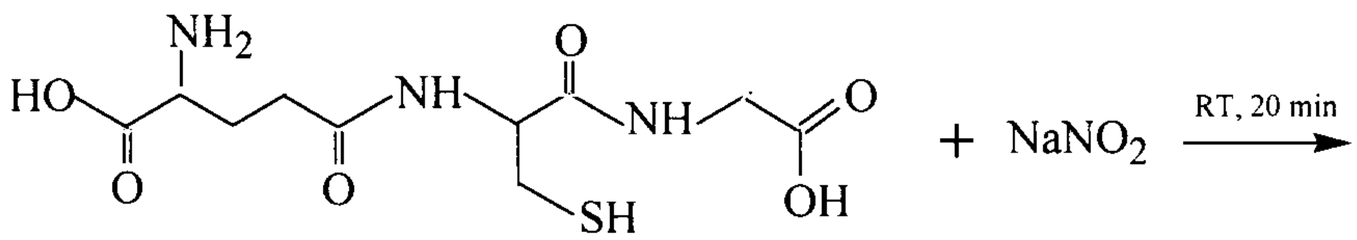
DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Experimental Methods

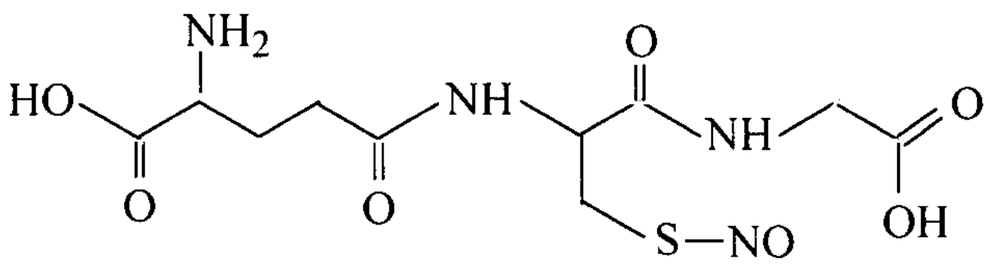
Preparation of GSNO-PVMMA-PVP Supramacromolecular Complex

Hydrophobic polyanhydride, poly(vinyl methyl ether-*alt*-maleic anhydride) (PVMMA) was selected as a nitric oxide carrier. A characteristic S-nitrosothiol compound, S-nitrosoglutathione (GSNO) was selected as the nitric oxide donor. The synthesis of GSNO conjugated PVMMA was performed as follows.

GSNO was first obtained through a rapid reaction between glutathione and sodium nitrite in aqueous solution protected from exposure to light (Scheme 1).



GSH ($C_{10}H_{17}N_3O_6S$)



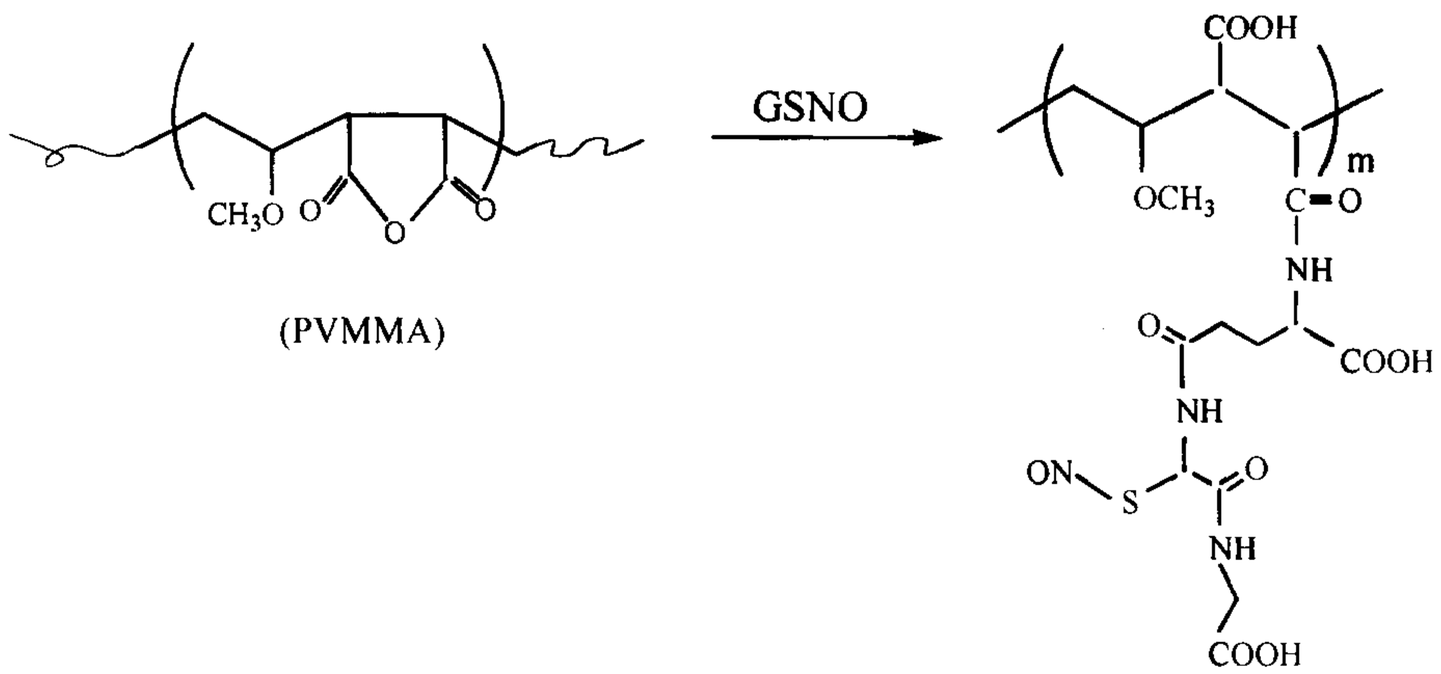
GSNO ($C_{10}H_{16}N_4O_7S$)

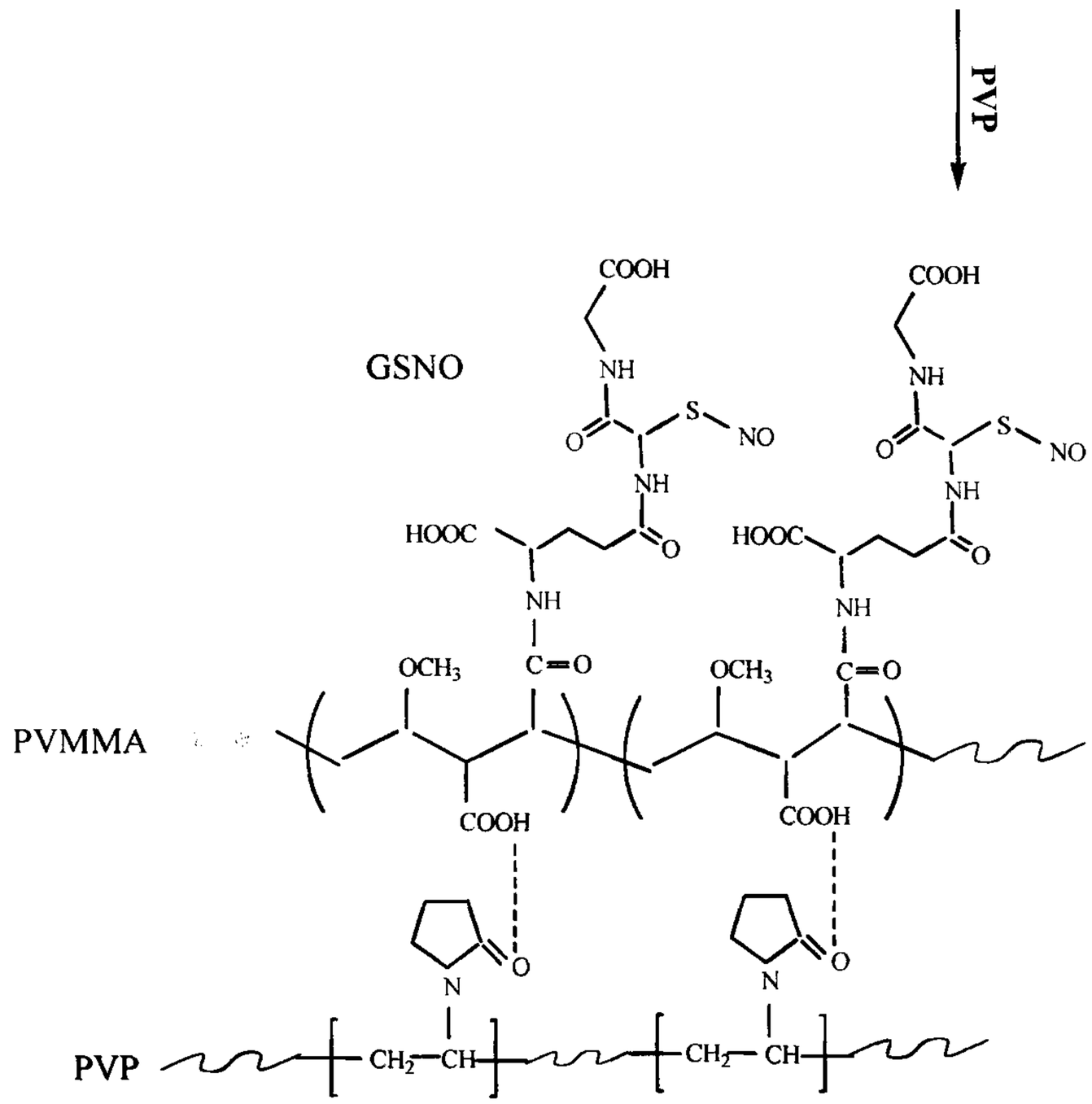
Scheme 1. S-nitrosation reaction of GSH, yielding GSNO.

Briefly, around 154 mg of glutathione (GSH) was allowed to react with 35 mg of $NaNO_2$ in 1 ml of deionized water and ethanol mixture (volume ratio = 1:1) under room temperature. The resultant pink GSNO solution was added drop-wise into 20 ml of a 2.5 wt% PVMMA solution in organic solvent such as dimethyl sulfoxide (DMSO), N, N-dimethylformamide (DMF) or N-methyl pyrrolidone (NMP), under vigorous stirring, to produce an initially pink emulsion. The emulsion became clear after continuous gentle stirring for an additional 20 min which, indicates that the coupling reaction between the

anhydride groups on PVMMA and the amino groups on GSNO was complete in forming GSNO-PVMMA. Such condensation reaction also resulted in the formation of free carboxylic acid groups in GSNO-PVMMA, which are essential in providing protons for the next step of formation of intermacromolecular complexes with PVP.

In the next step, to the stirred clear GSNO-PVMMA solution, was slowly added a PVP solution (5 wt% in ethanol) to form the desired supramacro intermolecular complex. Different volumes of the PVP solution were introduced to arrive at various desired PVMMA/PVP ratios in the final composition (Scheme 2).





Scheme 2. Synthetic steps and chemical structure of the GSNO-PVMMA/PVP supramacromolecular complex.

As complex formation took place, through intermolecular hydrogen bonding, the viscosity of the resultant mixture showed a distinct increase, giving rise to a pink gel-like product with the gelation degree varying with the ratio of PVMMA and PVP. The resulting semi-solid product was then transferred into an excess of ethyl ether to precipitate the polymer complex. After the pink polymer complex completely solidified from ethyl ether, it was collected by filtration, and the trace amount of organic solvent was removed under vacuum for 2 hours at room temperature. The brittle product so obtained was milled into powder in a Micro-Mill™ laboratory grinding mill and stored in an amber container in a dessicator prior to use.

Characterization of GSNO-PVMMA-PVP Supramacromolecular Complex

The hydrogen bonding interaction between PVMMA and PVP was characterized by Fourier transform infrared (FTIR) and the spectra recorded on a universal Attenuated Total

Reflectance (ATR) Spectrum-one™ Perkin-Elmer spectrophotometer (Perkin Elmer, Connecticut, USA). All spectra were collected from a patch of PVMMA, PVP and PVMMA/PVP complex at a resolution of 2 cm^{-1} and were repeated three times. A background spectrum without any sample was subtracted from all spectra. The spectra were recorded from 4000 ~ 650 cm^{-1} (see Fig. 1 & Fig. 2).

Characterization of NO Release

The *in vitro* release experiments of NO from GSNO-PVMMA/PVP were carried out at 37 and 25 °C by suspending 20 mg of polymer powder in 10 ml of 0.1 M PBS (pH = 7.4) in a scintillation vial. The amount of NO released over time was detected by the Griess Method, wherein, briefly, 1 ml of Griess reagent (NEDD) (0.1% w/v) plus 1 ml of sulfanilamide (1% w/v in 5% v/v H_3PO_4) at room temperature was incubated with an equal volume (2 ml) of sample for 20 minutes. UV absorbance at 540 nm wavelength was determined using a Cary 50 UV-Vis spectrophotometer (Varian, Ontario, Canada), and the total [NO_2^-] in the solution was calculated from the standard curve of 3-120 µmol/L $NaNO_2$, and the results were expressed as µmol.

Results and Discussion

In the aforesaid process, according to the invention, for making the supramacromolecular complex, GSNO is immobilized by covalently grafting it onto PVMMA to form GSNO-PVMMA and subsequently forming a supramacromolecular complex with PVP. Because of the hydrophobic nature of PVMMA and the strong hydrogen bonding of GSNO-PVMMA with PVP, the liberation of NO from the immobilized GSNO is controlled by the surface erosion characteristics of the present supramacromolecular complex system. Without forming the supramacromolecular complex with PVP, the release of NO from GSNO-PVMMA is relatively rapid in providing a release period only up to 3 days as evident in Fig. 3. On the other hand, nitric oxide release rate can be significantly slowed down after the formation of supramacromolecular complex with PVP due to its decreased dissociation rate in an aqueous medium. A typical profile of such NO release with a release duration lasting over 9 days is shown in Fig. 4. Where the in vitro release of NO in phosphate buffer saline at 25 °C from different GSNO-PVMMA/PVP supramacromolecular complex compositions containing 16.6 wt% of GSNO and different PVMMA/PVP weight ratios is presented. It is clear from Fig. 4 that the release rate of NO increases with the

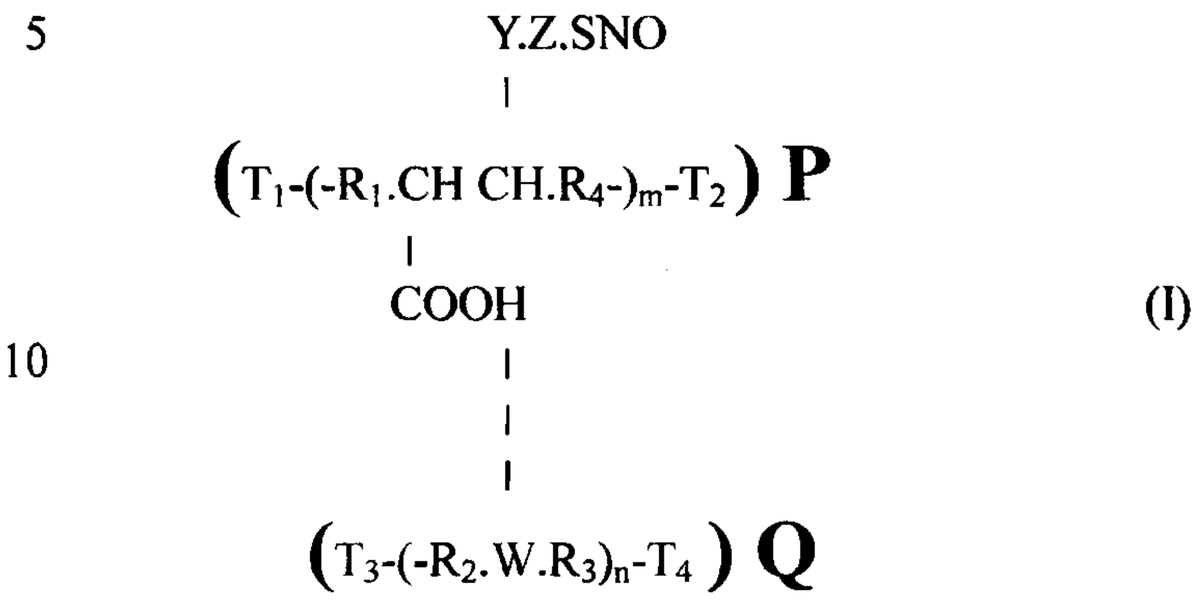
concentration of the hydrophilic component PVP in the supramacromolecular complex. The release rate of NO is also temperature dependent with a faster release rate at a higher temperature as shown in Fig. 5. As nitric oxide is gradually liberated from the complex, more disulfide bonds will form, giving rise to in-situ disulfide crosslinking between GSNO side chains which further reinforces the network structure of the complex. Based on the polymer structure and state of chain packing, different sustained and controllable release rate can be obtained by adjusting the component polymer molecular weight and concentration ratio, as well as the precipitation condition. Examples showing the effect of polymer molecular weight of PVMMA and PVP on the NO release behavior in the present supramacromolecular complex system are presented in Figs. 6A and 6B. It is evident from Figs. 6A and 6B that a smaller molecular weight of either PVMMA or PVP will result in a faster NO release.

In addition to poly(methyl vinyl ether-co-maleic anhydride) described in the above examples, applicable variations of this polymer component in the present invention also include maleic anhydride polymer and copolymers such as poly(methyl vinyl ether-alt-maleic anhydride), poly(maleic acid-co-maleic anhydride), poly(maleic anhydride), poly(vinylpyrrolidone-co-dimethyl maleic anhydride), poly(vinylacetate-co-maleic anhydride), poly(ethylene-alt-maleic anhydride), poly(isobutylene-alt-maleic anhydride), poly(styrene-alt-maleic anhydride), poly(ethylene-co-ethyl acrylate-co-maleic anhydride), and poly(maleic anhydride-alt-1-octadecene), and the like as well as their derivative thereof. Similarly, in addition to incorporating S-nitrosoglutathione, other applicable nitric oxide donors include S-nitrosothiols of cysteine, γ-Glu-Cys, α-Glu-Cys, glutathione, homoglutathione, hydroxymethyl-glutathione, γ-Glu-Cys-Glu, α-Glu-Cys-Gly, α-Glu-Cys-β-Ala, α-Glu-Cys-Ser, α-Glu-Cys-Glu, other glutathione analog containing –SH and $–NH_2$ and/or –OH functional groups, or one of the following peptides: $(\gamma\text{-Glu-Cys})_q$, $(\gamma\text{-Glu-Cys})_q$-Gly, $(\gamma\text{-Glu-Cys})_q$-β-Ala, $(\gamma\text{-Glu-Cys})_q$-Ser, $(\gamma\text{-Glu-Cys})_q$-Glu, $(\alpha\text{-Glu-Cys})_q$, $(\alpha\text{-Glu-Cys})_q$-Gly, $(\alpha\text{-Glu-Cys})_q$-β-Ala, $(\alpha\text{-Glu-Cys})_q$-Ser, and $(\alpha\text{-Glu-Cys})_q$-Glu, where q=2-7, and the like as well as their derivative thereof.. Similarly, in addition using poly(vinyl pyrrolidone) as the second polymeric component of the supramacromolecular complex of the present invention, other hydrogen bond accepting polymers, such as polyethylene glycol, poly(ethylene oxide), poly(vinyl pyrrolidone-co-vinyl acetate), polyethylene oxide-polypropylene oxide block copolymers (Pluronics or Polaxomers), polyethylene glycol fatty alcohol esters, polyethylene glycol fatty acids esters, and the like as well as their derivatives.

Although this disclosure has described and illustrated certain preferred embodiments of the invention, it is to be understood that the invention is not restricted to those particular embodiments. Rather, the invention includes all embodiments which are functional or mechanical equivalence of the specific embodiments and features that have been described and illustrated.

Claims:

1. A bio-adhesive supramacromolecular complex of the general formula (I):



(I)

wherein R_1 is an alkane unsubstituted or substituted with alkoxy groups;

R_2 is a lower alkane;

R_3 and R_4 are long chain, optionally substituted, alkanes;

W is a hydrogen-bond accepting functional group-containing entity;

Y is a carboxylic acid ester or amide;

Z is a linking group;

T_1, T_2, T_3 and T_4 are terminal groups;

m and n are integers selected from at least 25; and wherein P has a molecular weight of about 1×10^3 to 1×10^6 and Q has a molecular weight of about 1×10^3 to 1×10^7.

2. A supramacromolecular complex as claimed in claim 1 wherein R_1 is a maleic acid copolymer.

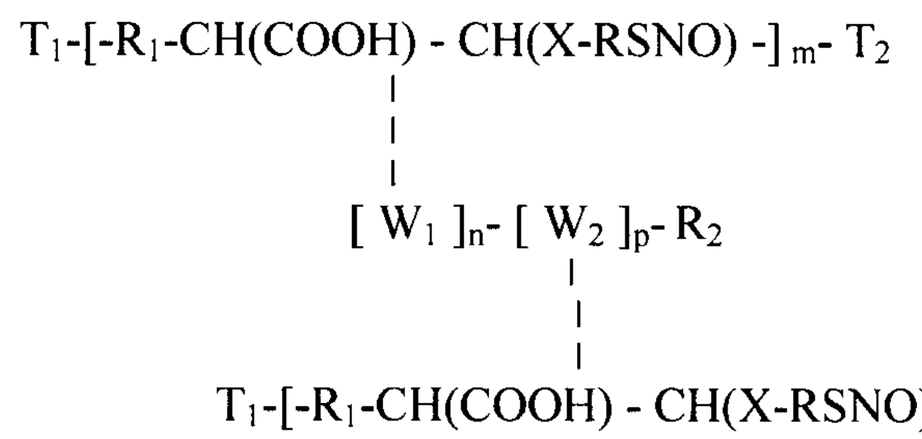
3. A supramacromolecular complex as claimed in claim 2 wherein said maleic acid copolymer is selected from the group consisting of poly(methyl vinyl ether-co-maleic acid) poly(vinylpyrrolidone-co-dimethyl maleic acid), poly(ethylene-co-maleic acid), poly(isobutylene-co-maleic acid), poly(styrene-co-maleic acid), poly(ethylene-co-ethyl acrylate-co-maleic acid), poly(maleic acid-co-octadecene), polyethylene-graft-maleic anhydride, polypropylene-graft-maleic acid, and polyisoprene-graft-maleic acid.

4. A supramacromolecular complex as claimed in any one of claims 1 to 3 wherein said $T_3(-R_2W.)_n-(R_3)_p-(-R_2W)_n-T_3$ is selected from the group consisting of poly(vinyl pyrolidone), polyethylene glycol, poly(ethylene oxide), poly(vinyl pyrrolidone-co-vinyl acetate), polyethylene oxide-polypropylene oxide block copolymers (Pluronics or Polaxomers), polyethylene glycol fatty alcohol esters, and polyethylene glycol fatty acids esters.

5. A supramacromolecular complex as claimed in claim 4 wherein said $T_3(-R_2W.)_n-(R_3)_p-(-R_2W)_n-T_3$ is poly(vinyl pyrolidone).

6. A supramacromolecular complex as claimed in any one of claims 1 to 5 wherein Y.Z.SNO is an amido-S-nitrosoglutathione.

7. A bio-adhesive supramacromolecular complex of the general formula (I):



wherein R_1 is an alkyl vinyl ether ($C_1 - C_5$), ethylene, propylene, isobutylene, butadiene, 1-octadecene, styrene, maleic acid, or maleic anhydride unit;

W_1 and W_2 are hydrogen-bond accepting functional group-containing entities selected from vinylpyrrolidone, ethylene oxide or propylene oxide, and vinyl acetate;

R_2 is H, a fatty acid ester, or fatty alcohol;

X is a carboxylic acid ester or amide linkage;

RSNO is a S-nitrosothiol of cysteine, γ-Glu-Cys, α-Glu-Cys, glutathione, homoglutathione, hydroxymethyl-glutathione, γ-Glu-Cys-Glu, α-Glu-Cys-Gly, α-Glu-Cys-β-Ala, α-Glu-Cys-Ser, α-Glu-Cys-Glu, other glutathione analog containing –SH and $–NH_2$ and/or –OH functional groups, or one of the following peptides: $(\gamma\text{-Glu-Cys})_q$, $(\gamma\text{-Glu-Cys})_q$-Gly, $(\gamma\text{-Glu-Cys})_q$-β-Ala,

(γ-Glu-Cys)$_q$-Ser, (γ-Glu-Cys)$_q$-Glu, (α-Glu-Cys)$_q$, (α-Glu-Cys)$_q$-Gly, (α-Glu-Cys)$_q$-β-Ala, (α-Glu-Cys)$_q$-Ser, and (α-Glu-Cys)$_q$-Glu, where q=2-7;

T_1 and T_2 are terminal groups;

n, m, and p are integers greater than 25.

8. A supramacromolecular complex as claimed in claim 7 wherein T_1-[-R_1-CH(COOH) -CH(X-RSNO) -]$_m$-T_2 is a reaction adduct of RSNO of claim 1 and a maleic anhydride polymer or copolymer.

9. A supramacromolecular complex as claimed in claim 8 wherein said maleic anhydride polymer or copolymer is selected from the group consisting of poly(methyl vinyl ether-alt-maleic anhydride), poly(maleic acid-co-maleic anhydride), poly(maleic anhydride), poly(vinylpyrrolidone-co-dimethyl maleic anhydride), poly(vinylacetate-co-maleic anhydride), poly(ethylene-alt-maleic anhydride), poly(isobutylene-alt-maleic anhydride), poly(styrene-alt-maleic anhydride), poly(ethylene-co-ethyl acrylate-co-maleic anhydride), and poly(maleic anhydride-alt-1-octadecene).

10. A supramacromolecular complex as claimed in claim 8 wherein said nitric oxide donor RSNO is selected from the group consisting of S-nitrosothiols of cysteine, γ-Glu-Cys, α-Glu-Cys, glutathione, homoglutathione, hydroxymethyl-glutathione, γ-Glu-Cys-Glu, α-Glu-Cys-Gly, α-Glu-Cys-β-Ala, α-Glu-Cys-Ser, α-Glu-Cys-Glu, other glutathione analog containing –SH and –NH_2 and/or –OH functional groups, or one of the following peptides: (γ-Glu-Cys)$_q$, (γ-Glu-Cys)$_q$-Gly, (γ-Glu-Cys)$_q$-β-Ala, (γ-Glu-Cys)$_q$-Ser, (γ-Glu-Cys)$_q$-Glu, (α-Glu-Cys)$_q$, (α-Glu-Cys)$_q$-Gly, (α-Glu-Cys)$_q$-β-Ala, (α-Glu-Cys)$_q$-Ser, and (α-Glu-Cys)$_q$-Glu, where q=2-7.

11. A supramacromolecular complex as claimed in any one of claims 7 to 10 wherein said [W_1]$_n$-[W_2]$_p$- R_2 is selected from the group consisting of poly(vinyl pyrrolidone), polyethylene glycol, poly(ethylene oxide), poly(vinyl pyrrolidone-co-vinyl acetate), polyethylene oxide-polypropylene oxide block copolymers (Pluronics or Polaxomers), polyethylene glycol fatty alcohols, and polyethylene glycol fatty acids esters.

12. A supramacromolecular complex as claimed in claim 11 wherein said [W_1]$_n$-[W_2]$_p$- R_2 is poly(vinyl pyrrolidone).

13. A supramacromolecular complex as claimed in any one of claims 7 to 12 wherein X-RSNO is an amido-S-nitrosoglutathione.

14. A method of making a bio-adhesive, supramacromolecular nitric oxide generatable polymer complex, said method comprising

(i) covalently linking a S-nitroso compound having an amino linking group with a bio-adhesive, hydrophobic polyanhydride compound to form a nitric oxide donor polymeric carrier; and

(ii) mixing said carrier with an hydrophilic intermolecular hydrogen bond-acceptable polymer to produce said supramacromolecular nitric oxide generatable complex.

15. A method as claimed in claim 14 wherein said S-nitrosocompound is mixed with said polyanhydride compound in an organic solvent at ambient temperature to effect a condensation reaction between the anhydride groups of said polyanhydride compound and amino linking groups of said S-nitroso compound to produce amido groups and free carboxylic acid groups in said polymeric carrier.

16. A process as claimed in claim 15 wherein said organic solvent is selected from dimethyl sulfoxide,N,N-dimethylformamide and N-methyl pyrolidone.

17. A method as claimed in any one of claims 14 to 16 wherein said S-nitroso compound is S-nitrosoglutathione (GSNO).

18. A method as claimed in any one of claims 14 to 17 wherein said polyanhydride compound is a maleic anhydride polymer or copolymer.

19. A method as claimed in claim 18 wherein said maleic anhydride copolymer is selected from the group consisting of poly(methyl vinyl ether-co-maleic anhydride) poly(vinylpyrrolidone-co-dimetyl maleic anhydride), poly(ethylene-co-maleic anhydride), poly(isobutylene-co-maleic anhydride), poly(styrene-co-maleic anhydride), poly(ethylene-co-ethyl acrylate-co-maleic anhydride), poly(maleic anhydride-co-octadecene), polyethylene-graft-maleic anhydride, polypropylene-graft-maleic anhydride, and polyisoprene-graft-maleic anhydride.

20. A method as claimed in claim 19 wherein said maleic anhydride copolymer is poly(methyl vinyl ether-co-maleic anhydride).

21. A method as claimed in any one of claims 14 to 20 wherein said hydrogen bond acceptable polymer is selected from the group consisting of poly(vinyl pyrolidone),

polyethylene glycol, poly(ethylene oxide), poly(vinyl pyrrolidone-co-vinyl acetate), polyethylene oxide-polypropylene oxide block copolymers (Pluronics or Polaxomers), polyethylene glycol fatty alcohol esters, and polyethylene glycol fatty acids esters.

22. A method as claimed in claim 21 wherein said hydrogen bond acceptable polymer is poly(vinyl pyrolidone).

23. A bio-adhesive, supramacromolecular nitric oxide generatable complex when made by a method as claimed in any one of claims 14 to 22.

24. A pharmaceutical composition comprising an effective wound healing amount of said supramacromolecular complex as claimed in any one of claims 1 to 13, or 23 and a physiological acceptable carrier.

25. A supramacromolecular complex as claimed in any one of claims 1 to 13 and 23 or a composition as claimed in claim 24, in the physical form of a powder, spun fiber, or coating on a surface of a substrate.

26. A supramacromolecular complex as claimed in claim 25 wherein said substrate is a catheter or stent.

27. A skin covering for application to the skin, said covering incorporating an effective wound healing amount of a supramacromolecular complex as claimed in any one of claims 1 to 13, or 23, or a composition as claimed in claim 24.

28. A skin covering as claimed in claim 27 in the form of a bandage or wound dressing.

29. A method of enhancing the healing of a skin wound or infection comprising applying an effective wound or infection healing amount of a supramacromolecular complex as claimed in any one of claims 1 to 13, or 23, or a composition as claimed in claim 24, to said wound.

30. Use of a supramacromolecular complex as claimed in any one of claims 1 to 13, or 23, or a pharmaceutically acceptable composition, thereof, as claimed in claim 24, for enhancing the healing of a skin wound or infection.

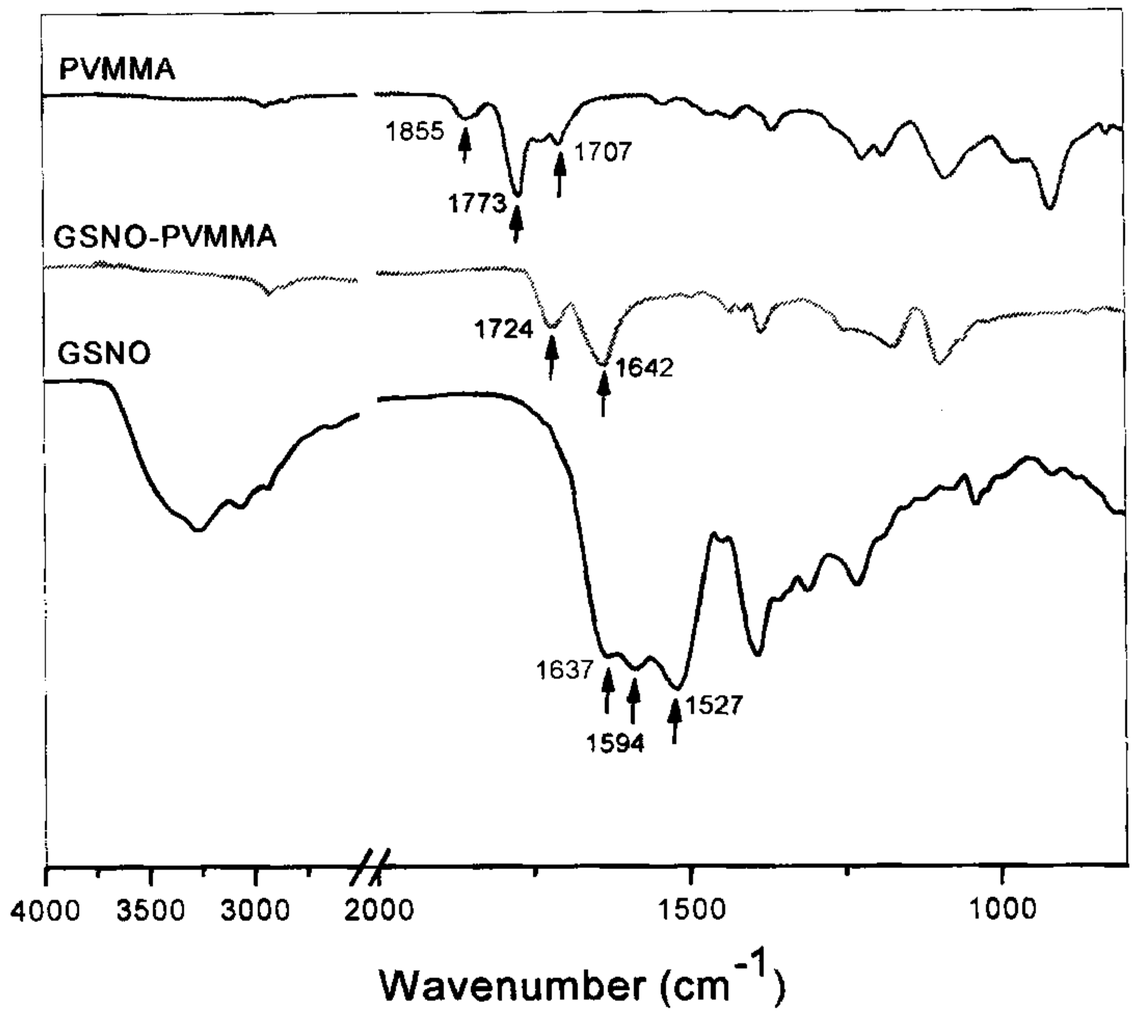

PVMMA
1855
1707
1773
GSNO-PVMMA
1724
1642
GSNO
1637
1527
1594
4000
3500
3000
2000
1500
1000
Wavenumber (cm-1)

FIG. 1

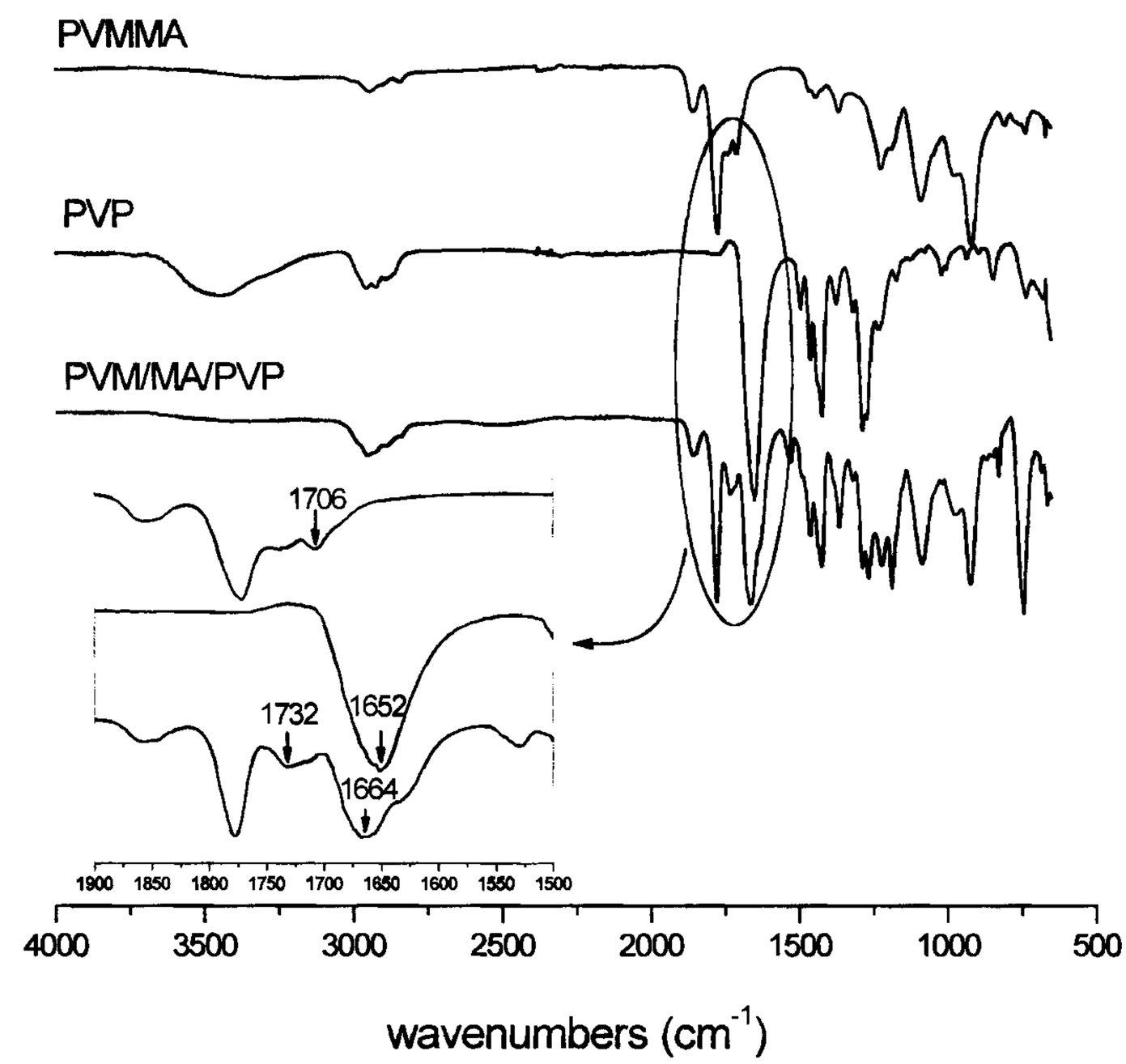

PVMMA
PVP
PVM/MA/PVP
1706
1732
1652
1664
1900
1850
1800
1750
1700
1650
1600
1550
1500
4000
3500
3000
2500
2000
1500
1000
500
wavenumbers (cm-1)

FIG.2

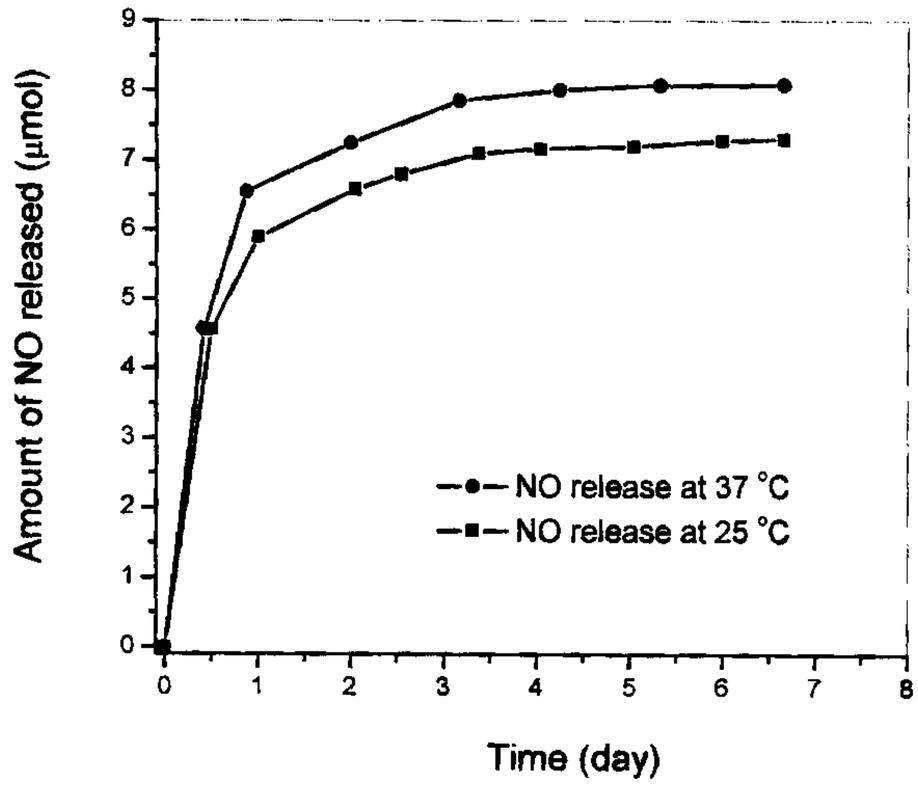


FIG. 3

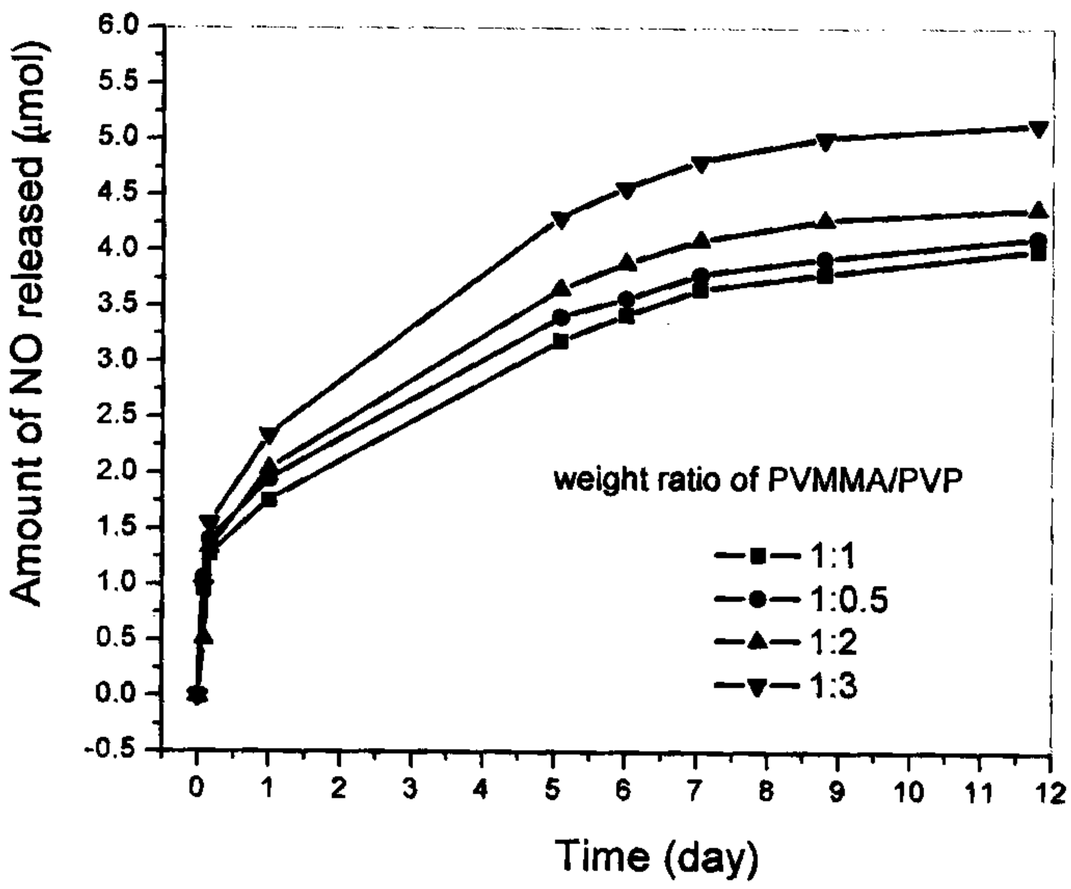


FIG.4

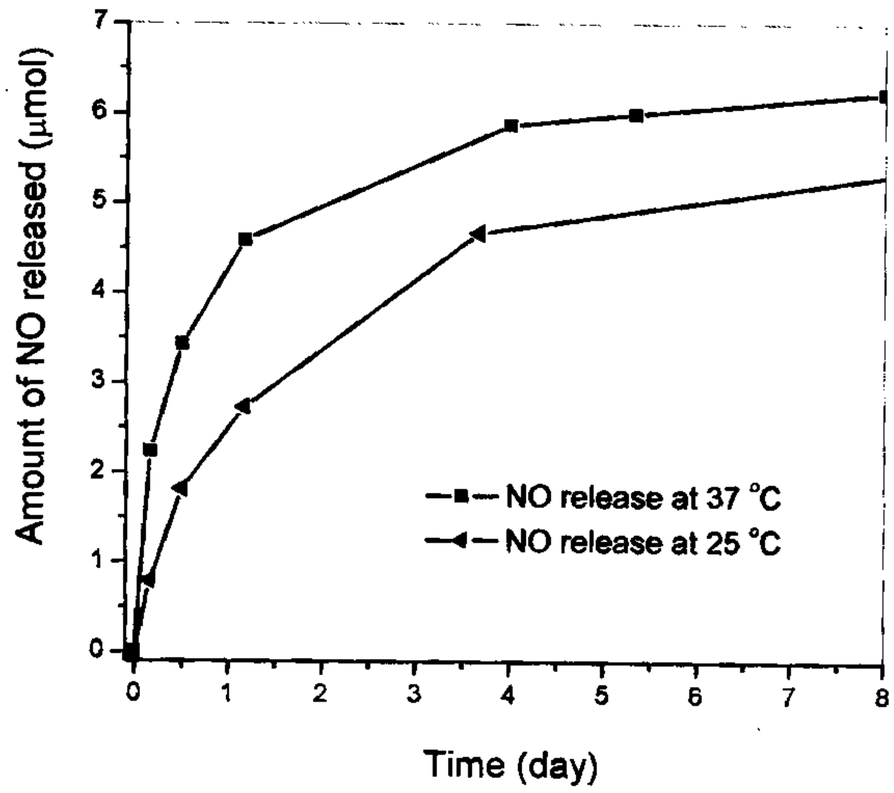


FIG.5

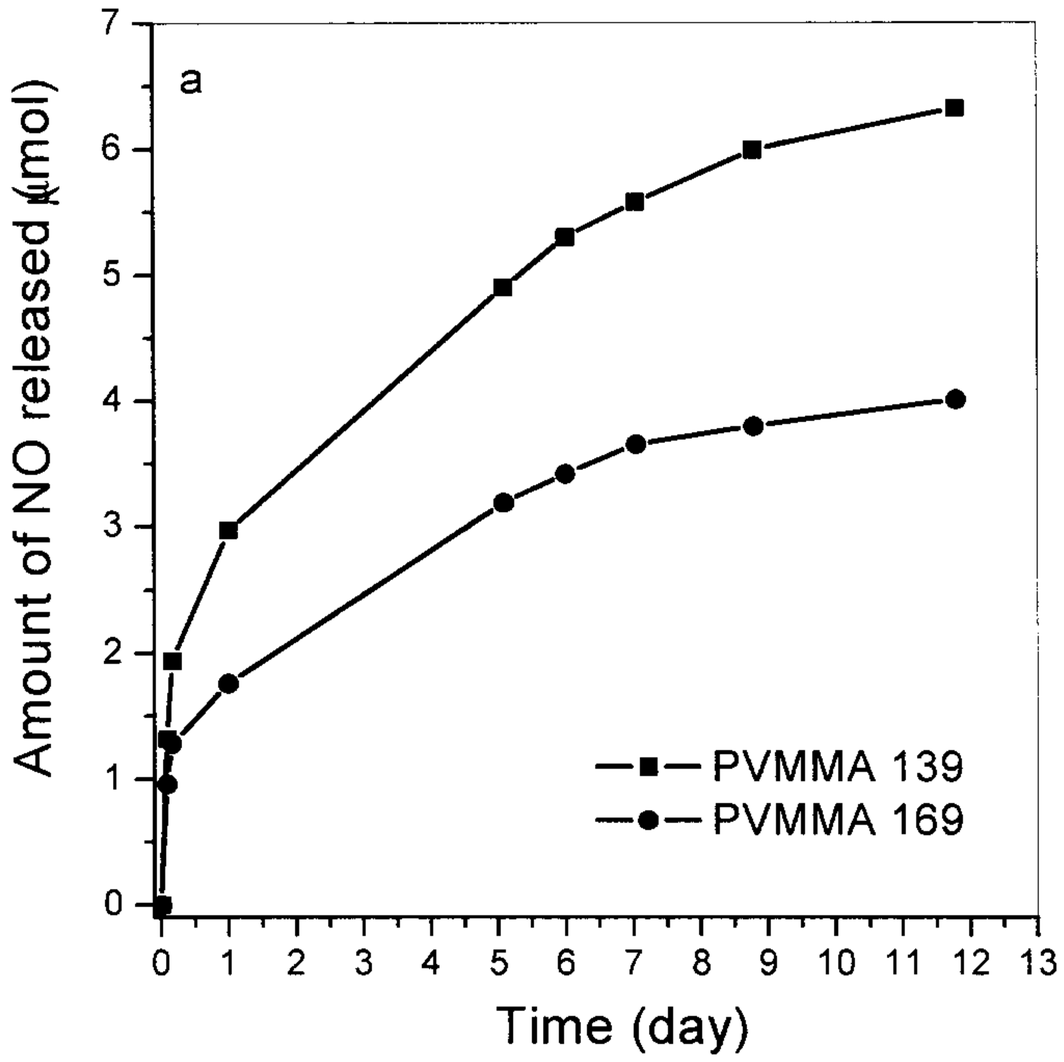

a
Amount of NO released (μmol)
Time (day)
PVMMA 139
PVMMA 169
0
1
2
3
4
5
6
7
0 1 2 3 4 5 6 7 8 9 10 11 12 13

FIG. 6A

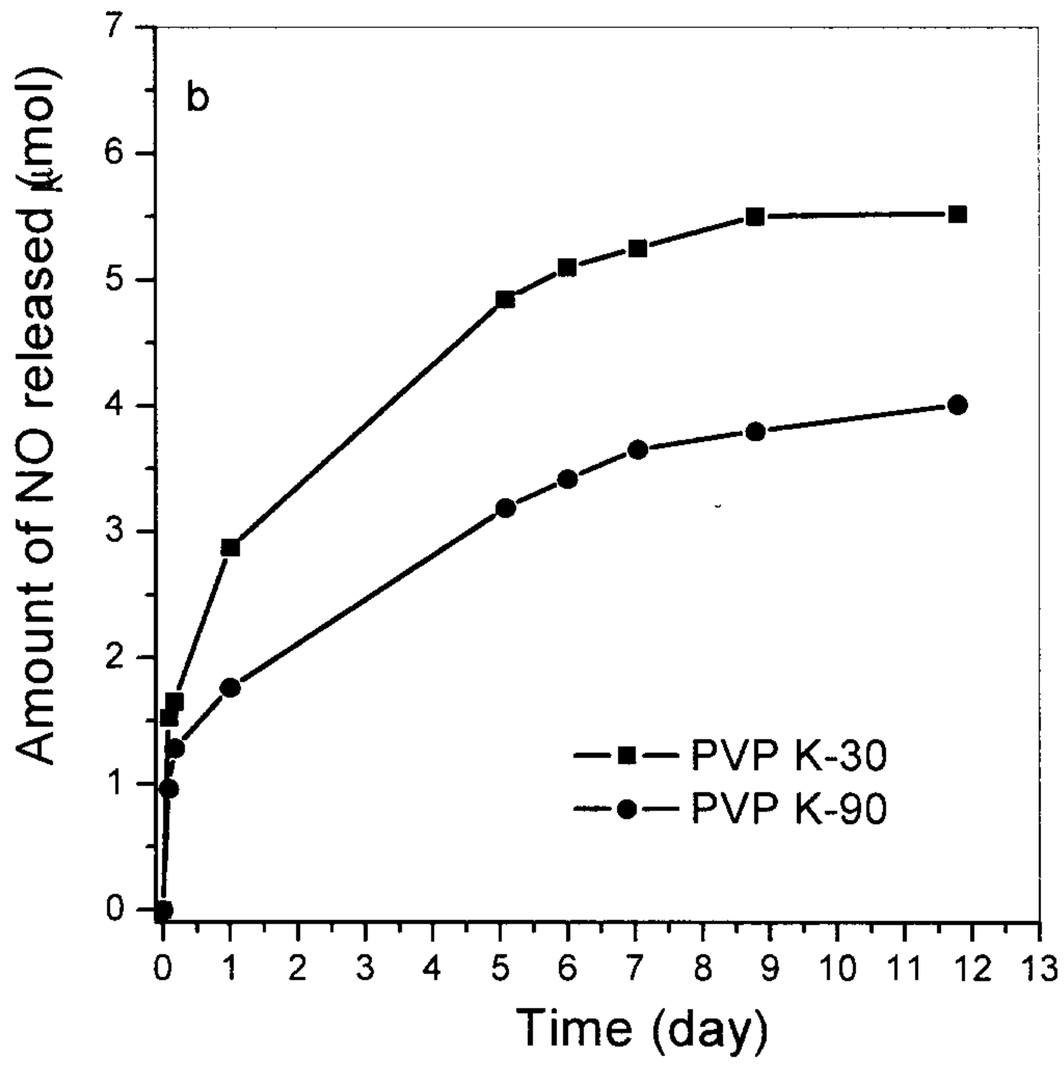
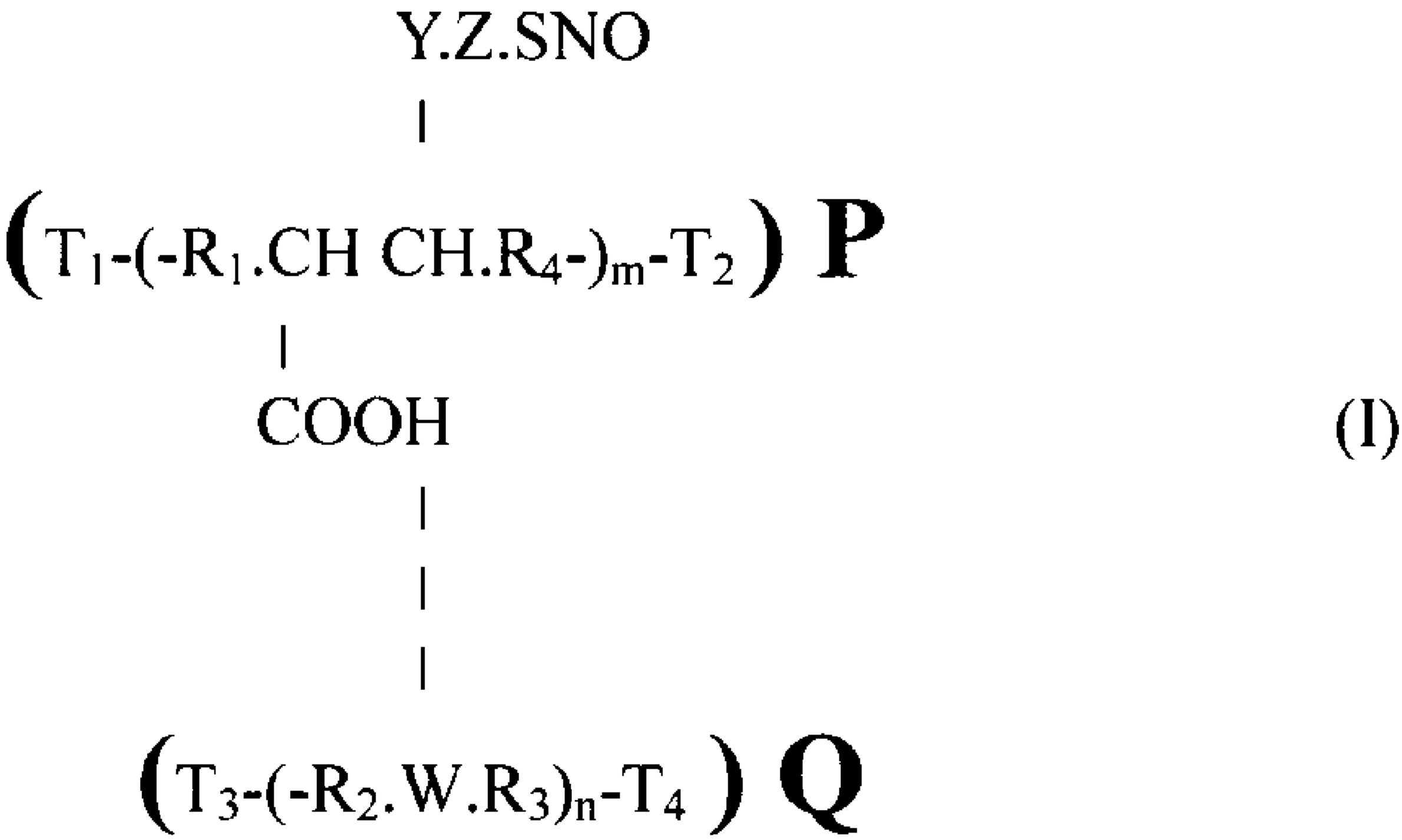

b
Amount of NO released (μmol)
Time (day)
PVP K-30
PVP K-90
0
1
2
3
4
5
6
7
0 1 2 3 4 5 6 7 8 9 10 11 12 13

FIG. 6B



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