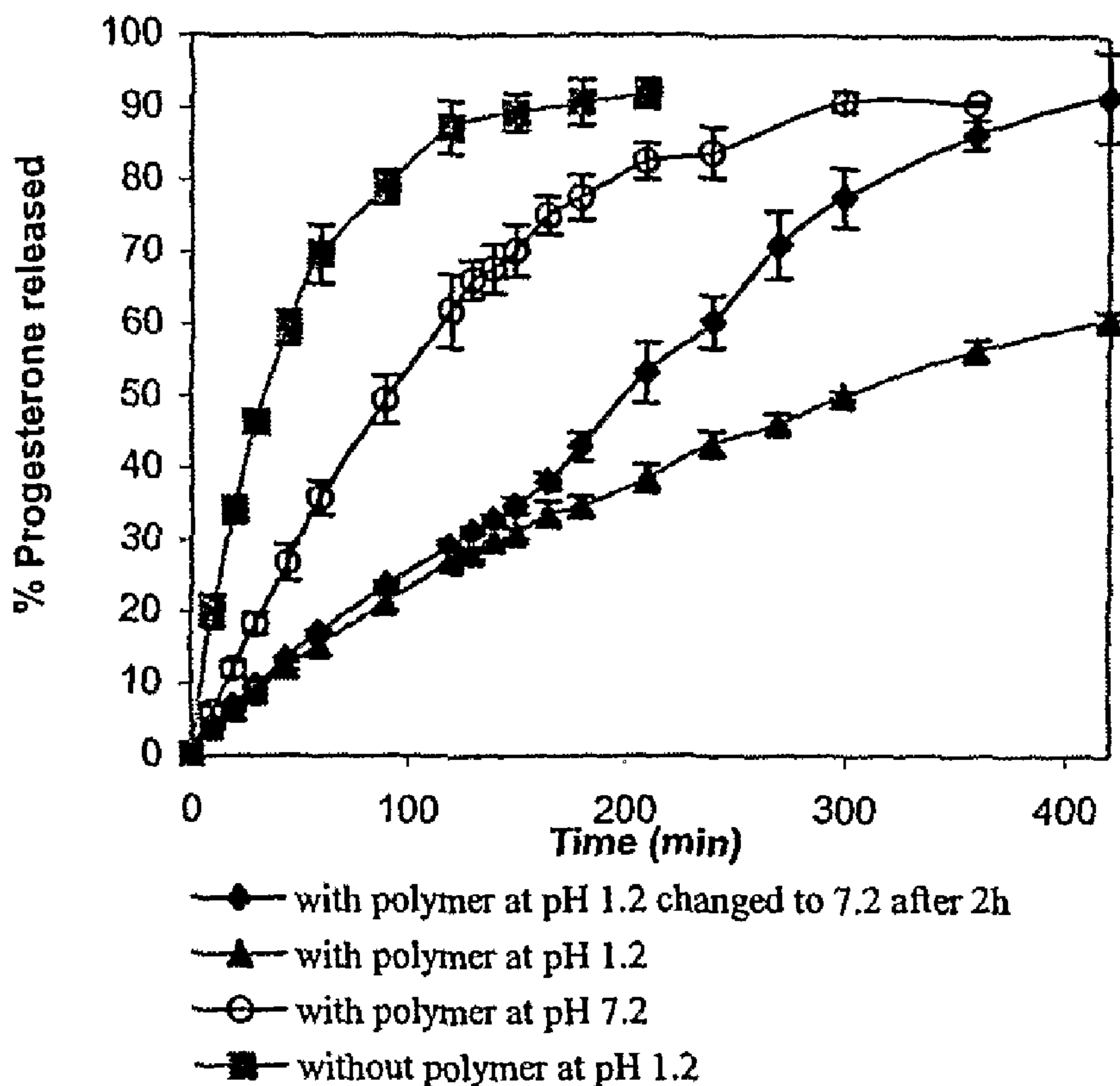




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 (71) Demandeur/Applicant:
 UNIVERSITE DE MONTREAL, CA
 (72) Inventeurs/Inventors:
 SANT, VINAYAK, CA;
 LEROUX, JEAN-CHRISTOPHE, CA
 (74) Agent: MARCOUX, PAUL

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 (54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING pH-SENSITIVE BLOCK COPOLYMERS AND A HYDROPHOBIC DRUG



(57) Abrégé/Abstract:

The present invention relates to a pharmaceutical composition of a biologically active agent and a block copolymer composed of a poly(ethylene oxide) forming hydrophilic segment and a poly(butyl (alkyl)acrylate-co-(alkyl)acrylic acid) that is capable of forming

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supramolecular assemblies or micelles under favourable conditions. The supramolecular assemblies or micelles formed from said polymers associate or dissociate reversibly upon changes in the environmental pH. The pharmaceutical compositions of the present invention contain hydrophobic drugs, cations or polycationic compounds, which can be delivered to the body by oral route or other routes of administration.

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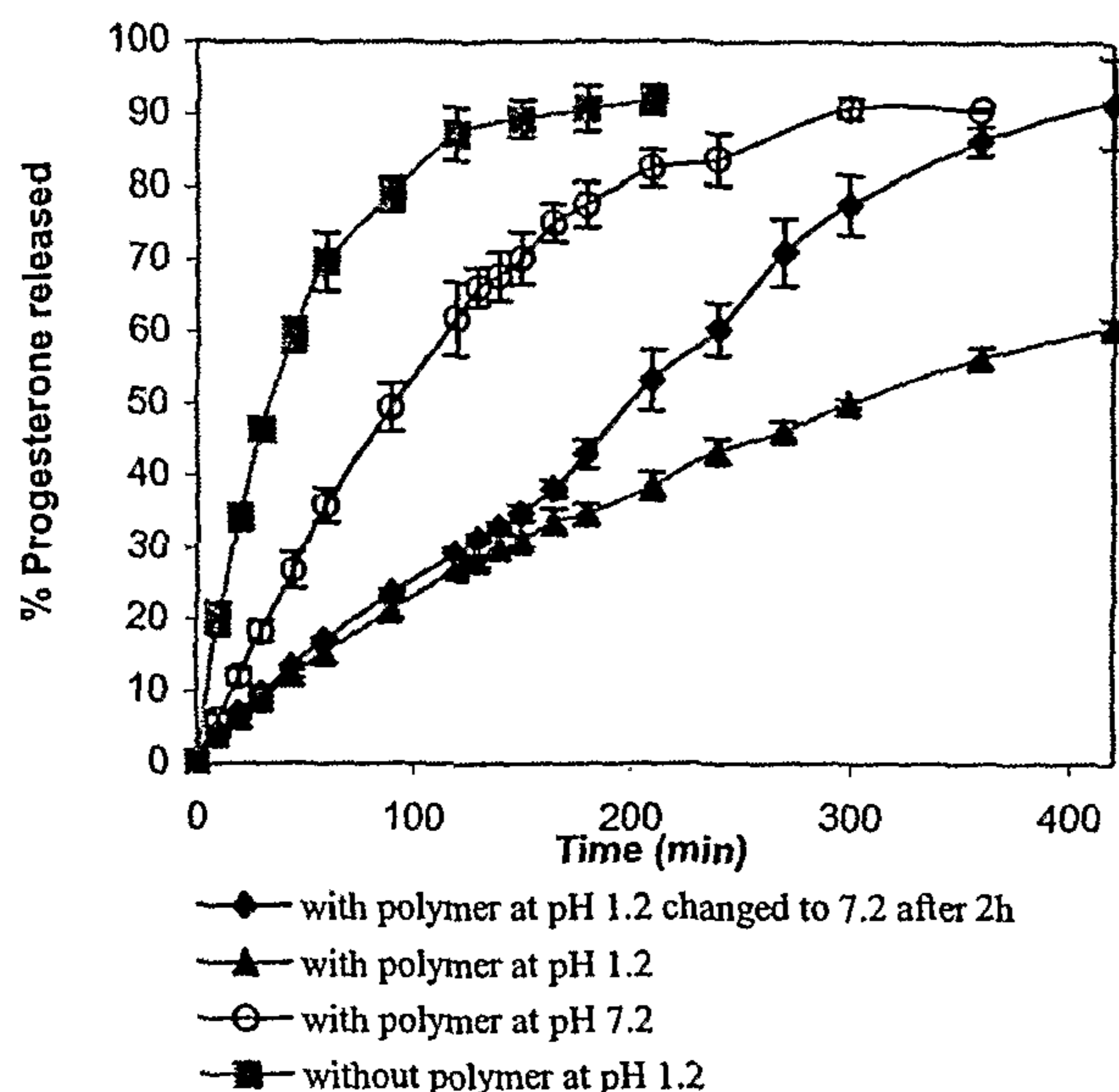
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- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **SANT, Vinayak** [CA/CA]; 7171 rue Waverly, Montreal, Quebec H2S 3J1 (CA). **LEROUX, Jean-Christophe** [CA/CA]; 329 Notre-Dame Est, Apt. 327, Montreal, Quebec H2Y 3Z2 (CA).
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(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING PH-SENSITIVE BLOCK COPOLYMERS AND A HYDROPHOBIC DRUG



(57) Abstract: The present invention relates to a pharmaceutical composition of a biologically active agent and a block copolymer composed of a poly(ethylene oxide) forming hydrophilic segment and a poly(butyl (alkyl)acrylate-co-(alkyl)acrylic acid) that is capable of forming supramolecular assemblies or micelles under favourable conditions. The supramolecular assemblies or micelles formed from said polymers associate or dissociate reversibly upon changes in the environmental pH. The pharmaceutical compositions of the present invention contain hydrophobic drugs, cations or polycationic compounds, which can be delivered to the body by oral route or other routes of administration.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

1 PHARMACEUTICAL COMPOSITIONS COMPRISING PH-SENSITIVE BLOCK COPOLYMERS AND A
2 HYDROPHOBIC DRUG

3 **REFERENCE TO RELATED APPLICATION**

4 This application is a continuation-in-part of pending U.S. S.N. 09/877,999, filed
5 June 8, 2001, the contents of which is incorporated herein by reference, in its entirety.

6 **FIELD OF THE INVENTION**

7 The present invention relates to stimuli responsive amphiphilic polymers forming
8 supramolecular assemblies or micelles in the nanometric size range under favourable
9 conditions. These supramolecular assemblies or micelles can be useful for the oral or
10 parenteral delivery of hydrophobic or cationic pharmaceutical agents.

11 **BACKGROUND OF THE INVENTION**

12 Amphiphilic block copolymers having optimal hydrophilic and hydrophobic
13 segments self-assemble spontaneously in aqueous environment forming micelles or
14 supramolecular assemblies. These supramolecular assemblies exhibit core-shell
15 architecture wherein the hydrophobic part forms the core and the hydrophilic part forms
16 the corona. Recently, polymeric micelles have been widely used as drug delivery carriers
17 for parenteral administration. Micellar drug delivery carriers have several advantages
18 including biocompatibility, solubilization of hydrophobic drugs in the core, nanometric
19 size ranges which facilitate extravasation of the drug carrier at the site of inflammation,
20 site-specific delivery etc. (see for example Torchilin VP, J Controlled Release, 2001, 73,
21 137-172; Kataoka et al, Adv Drug Deliv Rev, 2001, 47, 113-131; Jones et al, Eur J Pharm

1 Biopharm, 1999, 48, 101-111).

2 A large number of amphiphilic block copolymers, having nonionic and/or charged
3 hydrophobic and hydrophilic segments, that form micelles are reported in the literature.
4 Examples of some widely used block copolymers for parenteral delivery include
5 poly(ethylene oxide)-*b*-poly(D,L-lactide), poly(ethylene oxide)-*b*-poly(ϵ -caprolactone),
6 poly(ethylene oxide)-*b*-poly(aspartic acid), poly(N-vinyl pyrrolidone)-*b*-poly(D,L-lactide)
7 etc.

8 US Patent 6,322,805 describes polymeric drug carrier micelles prepared from
9 amphiphilic block copolymer having a hydrophilic poly(alkylene oxide) component and a
10 biodegradable hydrophobic component selected from a group consisting of poly(lactic
11 acid), poly(lactic-co-glycolic acid), poly(ϵ -caprolactone) and a derivative thereof. These
12 micelles are capable of solubilizing hydrophobic drug in a hydrophilic environment.

13 US Patent 6,338,859 describes polymeric micelle compositions where the
14 hydrophilic component includes poly(N-vinyl-2-pyrrolidone) and the hydrophobic
15 component is selected from a group consisting of polyesters, polyorthoesters,
16 polyanhydride and derivatives thereof. The polyester group can be selected from poly(D,L-
17 lactic acid), poly(glycolic acid), lactide/glycolide copolymers, poly(ϵ -caprolactone) and
18 derivatives thereof. The micelle composition contains a therapeutic agent which can be an
19 antitumor compound, hydrophobic antibiotic, hydrophobic antifungal agent, an
20 immunomodulator, an antiviral drug, or the like.

21 US Patent 6,383,811 describes formation of complexes of polyions such as DNA
22 with polyampholytes i.e. polymers possessing both cationic and anionic moieties, and
23 delivery of the complex into the cell.

1 US Patent 6,210,717 describes a composition composed of mixed polymeric
2 micelles made of amphiphilic polyester-polycation copolymer and an amphiphilic
3 polyester-sugar copolymer for delivery of nucleic acids into targeted host cells. The
4 polyester-polycation forms an electrostatic interaction with polyanionic nucleic acids, and
5 the polyester-sugar copolymer directs the micelle-nucleic acid complex to cells in vivo.

6 US Patent 6,429,200 describes delivery of polynucleotides to cells using cleavable
7 reverse micelles. Other molecules such as polymers, and surfactants containing disulphide
8 linkages can be included into the complex micelles to enhance the delivery.

9 US Patent 5,510,103 describes block copolymers having the hydrophilic and
10 hydrophobic segments forming micelles and entrapping the hydrophobic drugs by physical
11 methods. The hydrophilic segment is preferably poly(ethylene oxide) and hydrophobic
12 segment is preferably poly(β -benzyl -L-aspartate) while the preferred drug is adriamycin.

13 US Patent 5,955,509 describes use of poly(vinyl-N-heterocycle)-b-poly(alkylene
14 oxide) copolymers in micelle containing pharmaceutical formulations. These copolymers
15 respond to pH changes in the environment and can be used to deliver therapeutic
16 compounds at lower pH values. The micelles of these polymers remain intact at neutral
17 pH, e.g. at physiological pH, while they will release the contents when exposed to a lower
18 pH environment such as in the tumor.

19 US Patent 6,497,895 describes hyperbranched micelles containing a core of mucic
20 acid esters for the encapsulation of hydrophobic molecules. These polymers are useful for
21 the transdermal delivery of the entrapped agent in a controlled manner.

22 US Patent 6,387,406 describes compositions of the poly(oxyethylene)-
23 poly(oxypropylene) block copolymers for oral delivery of biological agents.

1 Nishiyama et al (Pharm Res 2001, 18, 1035-1041 ; J Controlled Release 2001, 74,
2 83-94) have described the use of poly(ethylene oxide)-b-poly(α,β -aspartic acid) block
3 copolymers forming micelles by interaction with an antitumor drug, specifically cisplatin.

4 Though the majority of these polymers can be used for oral delivery of bioactive
5 agents, what is presently lacking are amphiphilic polymers capable of forming
6 supramolecular assemblies that respond to an environmental stimuli such as pH change,
7 thereby entrapping the contents in the micelle core at a low pH, such as that prevailing in
8 the stomach, and rapidly releasing the contents at a higher pH, such as that prevailing in
9 the intestine.

10 In our earlier filed US patent application (S.N. 09/877,999, June 8, 2001) we
11 describe a series of ionizable diblock copolymers useful for the delivery of bioactive
12 agents. A series of the polymers in this patent application partially fulfills the above
13 requirement. These polymers are different from those disclosed in US Patent 5,955,509 in
14 that they form supramolecular assemblies at low pH, that could be dissociated upon
15 increase in the pH above pKa of the carboxyl group. Another characteristic of these
16 polymers is the presence of nonionizable and reversibly ionizable groups in the
17 hydrophobic segment, where, hydrophobicity can be changed by controlling the ionization.

18 **SUMMARY OF THE INVENTION**

19

20 The present invention relates to polymers useful in combination with
21 pharmaceutical compositions containing at least one biologically active agent. More
22 particularly, the invention relates to block copolymers having hydrophilic and hydrophobic
23 segments suitable for, but not limited to, oral drug delivery. More particularly, the

1 hydrophilic segment of the polymers is nonionic and the hydrophobic segment contains at
2 least one reversibly ionizable pendant carboxyl group conferring pH-sensitivity to the
3 polymers.

4 Accordingly, it is a primary objective of the instant invention to provide a
5 copolymer which is composed of a hydrophilic segment made of poly(ethylene oxide) and
6 a hydrophobic segment composed of vinyl monomers containing at least one pendant
7 carboxyl group. More particularly, the vinyl monomers included in the polymer are acrylic
8 acid or methacrylic acid having pendant carboxyl groups and butyl (alkyl)acrylate where
9 the butyl segment can be a linear or branched chain. Thus, the hydrophobic segment is a
10 mixture of non-ionizable butyl (alkyl)acrylate and ionizable (alkyl)acrylic acid which
11 controls the hydrophobicity of the polymer.

12 Another objective of the instant invention is to prepare pharmaceutical
13 compositions from the instantly disclosed polymers by entrapping at least one substance,
14 preferably a biologically active agent, which is illustrated by, albeit not limited to a
15 hydrophobic molecule, a cationic compound or macromolecule such as peptides and
16 proteins bearing cationic residues. The entrapment can be physical (e.g. hydrophobic
17 interaction, electrostatic interaction), or chemical (e.g. covalent linkage) in nature.

18 A further objective of the present invention is to prepare supramolecular assemblies
19 having core-shell structure wherein the core is formed by the hydrophobic segment, which
20 can reversibly dissociate and associate in response to a change in environmental pH
21 because of the pendant carboxyl group. The size of these supramolecular assemblies can
22 be between 5 to 1000 nanometers thereby forming a solution or colloidal dispersion in
23 water. It is to be noted that in the further text terms "micelles" and "supramolecular

1 assemblies” are used interchangeably and essentially mean structures having a size range
2 of between about 5 to 1000 nanometers.

3 Yet another objective of the instant invention is to describe methods of entrapping
4 the hydrophobic agents and cationic molecules in the supramolecular assemblies giving
5 high incorporation efficiencies.

6 A still further objective of the present invention is to use these supramolecular
7 assemblies for delivery of a bioactive agent into the body by, but not limited to, the oral
8 route. Upon oral administration, the hydrophobic molecule trapped in the core of the
9 supramolecular assembly will be protected from the harsh acidic conditions of the stomach
10 and released in the intestine due to dissociation of micelle at high pH.

11 Other objectives and advantages of this invention will become apparent from the
12 following description, inclusive of the experimental working examples, taken in
13 conjunction with the accompanying drawings wherein are set forth, by way of illustration
14 and example, certain embodiments of this invention. The drawings constitute a part of this
15 specification and include exemplary embodiments of the instant invention and illustrate
16 various objects and features thereof.

17 BRIEF DESCRIPTION OF THE FIGURES

18 Figure 1. demonstrates *in vitro* release of progesterone from PEO-b-poly(nBA₅₀-co-
19 MAA₅₀) supramolecular assemblies as a function of pH;

20 Figure 2: demonstrates the effect of pH on intensity of light scattered by solution of PEO-
21 b-poly(nBA₅₀-co-MAA₅₀);

22 Figure 3. is a plot of plasma concentration versus time of fenofibrate upon oral
23 administration of different formulations to Sprague-Dawley rats.

1

2 DETAILED DESCRIPTION OF THE INVENTION

3 Abbreviations:

4 nBA – n-butyl acrylate;

5 MAA – methacrylic acid;

6 EA – ethyl acrylate;

7 PEO – poly(ethylene oxide).

8 The present invention describes pharmaceutical compositions composed of block
9 polymers composed of a poly(ethylene oxide) forming the hydrophilic segment and a
10 poly(butyl (alkyl)acrylate-co-(alkyl)acrylic acid) forming the hydrophobic segment; and at
11 least one biologically active agent. The molecular weight of the hydrophilic segment can
12 be in the range of 200 to 80,000 Da, more preferably in the range of 500 to 10,000 Da, still
13 more preferably in the range of 2,000 to 5,000 Da. The hydrophobic segments of the
14 polymers in the present invention are composed of butyl (alkyl)acrylate and (alkyl)acrylic
15 acid, where the alkyl chain is composed of from 0 to about 10 carbon atoms, inclusively,
16 more preferably with 0 or 1 carbon atom. The butyl segment of the butyl (alkyl)acrylate
17 can be a linear or branched chain including without limitation n-butyl and tert-butyl
18 groups. The mole ratio of the butyl (alkyl)acrylate : (alkyl)acrylic acid in the hydrophobic
19 segment is in the range of about 5:95 to 95:5, more preferably in the range of about 30:70
20 to 70:30. The length of the hydrophobic segment can be in the range of about 200 to
21 50,000, more preferably in the range of about 500 to 80,000 Da.

22 Amphiphilic block copolymers have a tendency to self-assemble in water forming

1 micelles. Upon micellization, the hydrophobic segment forms a core and the hydrophilic
2 segment forms the corona of the micelles. The core of these micelles can be used as a
3 reservoir of hydrophobic compounds protecting them from the external environment. If
4 the hydrophobic segment of the polymer contains reversibly ionizable moieties, then the
5 hydrophobicity of the segment could be manipulated by controlling the ionization of the
6 moiety. Polymers of the present invention differ from other block copolymers in this
7 aspect. In the polymers of the present invention, the hydrophobic segment is composed of
8 the mixture of two monomers, one of them is butyl (alkyl) acrylate, which confers
9 hydrophobicity to the segment. Butyl (alkyl)acrylate monomer is more preferably butyl
10 acrylate or butyl methacrylate. The other monomer, (alkyl)acrylic acid has a pendant
11 carboxyl group that can be reversibly ionized by changing the environmental pH. Thus,
12 when the environmental pH is below the pKa of the carboxyl group, it will remain mostly
13 in the unionized form and will confer hydrophobicity to the segment. This results in
14 spontaneous aggregation of polymeric chains forming stable supramolecular assemblies or
15 micelles in aqueous environment. However, when environmental pH is increased above
16 pKa of the carboxyl group, its ionization will impart hydrophilicity to the hydrophobic
17 segment. This may result in the dissociation of the micelle. (Alkyl)acrylic acid monomer
18 is more preferably acrylic acid or methacrylic acid.

19 These supramolecular assemblies are in the size range of from about 5 to 1000
20 nanometers. Hydrophobic drugs are incorporated in the core of such supramolecular
21 assemblies by methods that are known to one of ordinary skill in the art (see for example
22 Lavasanifar et al J Controlled Release 2001, 77,155-60; Kohori et J Controlled Release
23 2002,78,155-63). Manipulation of the composition of the hydrophobic segment results in

1 variation in the hydrophobicity of the polymer allowing control of the incorporation
2 efficiencies of the hydrophobic drugs. Loadings in the range of 0.1 to 50% w/w more
3 preferably in the range of 1 to 20% w/w of hydrophobic drugs are obtained using different
4 drug loading procedures.

5 Block copolymers of the present invention are used to prepare pharmaceutical
6 compositions containing hydrophobic molecules. Non-limiting examples of the
7 hydrophobic molecules includes hypolipidemic agents such as fenofibrate, anticancer
8 agents such as doxorubicin, paclitaxel, docetaxel, camptothecin, megestrol acetate,
9 teniposide, etoposide, antihypertensive agents such as candesartan cilexetil, non-steroidal,
10 anti-inflammatory agents such as indomethacin, celecoxib, antiviral agents such as
11 retinovir, amprenavir, indinavir, efavirenz, immunosuppressive agents such as cyclosporin
12 A, sirolimus, tacrolimus, and similar agents belonging to other therapeutic classes.

13 In an alternative embodiment of the present invention, the poly(butyl
14 (alkyl)acrylate-co-(alkyl)acrylic acid) segment of the polymer will bear a negative charge
15 at a pH above the pKa of carboxyl groups and form complexes with cationic molecules
16 including without limitation polycations, peptides and proteins bearing cationic residues by
17 electrostatic interactions. This will result in the partial or complete charge neutralization of
18 polymer and/or cationic molecule thereby forming supramolecular assemblies or micelles.
19 The cation or polycationic molecule will be entrapped in the core of such supramolecular
20 assemblies. The term "cationic residues" refers to the functional groups imparting positive
21 charge to the molecule such as cationic amino acids e.g. lysine, arginine, histidine or other
22 functional groups such as primary, secondary, tertiary or quaternary amine groups present
23 in the molecule.

1 The complexes of poly(ethylene oxide)-block-poly(n-butyl acrylate-co-methacrylic
2 acid) with poly-l-lysine are prepared in a buffer solution at pH 7.4. Supramolecular
3 assemblies having unimodal size distribution within the size range of about 20 to 50 nm
4 are obtained depending upon the molecular weight of the poly-l-lysine and composition of
5 the polymer. On the other hand, complexes of poly(ethylene oxide)-block-poly(ethyl
6 acrylate-co-methacrylic acid) with poly-l-lysine in pH 7.4 buffer results in formation of
7 aggregates having multimodal size distribution with sizes above about 200 nm.

8 In yet another embodiment of the present invention, block copolymers are used to
9 form stable coordination complexes with biologically active agents illustrated as metallic
10 compounds such as cisplatin, carboplatin above the pKa of the carboxyl groups.

11 The presence of butyl (alkyl)acrylate in the hydrophobic segment plays several
12 crucial roles in forming stable supramolecular assemblies. It confers hydrophobicity to the
13 polymer chain, which is one of the important driving forces in the self-assembly of
14 polymeric chains. It also increases the incorporation of hydrophobic drugs in the
15 supramolecular assemblies. It is well known that carboxylic acid groups form intra- and/or
16 intermolecular hydrogen-bonding complexes with oxygen present in the polyethylene
17 oxide chain (see for example Donini et al, Int J Pharm, 2002,245, 83-91; Lele et al, J.
18 Controlled Release, 2000, 69, 237-248). This results in the formation of large aggregates or
19 sometimes in precipitation of the complex. This problem could be possible in
20 poly(ethylene oxide)-block-poly(aspartic acid) polymers (Nishiyama et al Pharm Res
21 2001, 18, 1035-1041; Yokoyama et al J Controlled release 1996, 39, 351-356). It was
22 evident in polymers having the composition poly(ethylene oxide)-block-poly(methacrylic
23 acid) as reported previously (Ranger et al J Polymer Science: part A: Polymer Chemistry,

1 2001, 39, 3861-3874). One method for overcoming this problem is by incorporating
2 hydrophobic monomers such as ethyl acrylate in the hydrophobic segment, as disclosed in
3 our earlier US patent application (09/877,999 June 8, 2001).

4 In accordance with the instantly disclosed invention, it was observed that polymers
5 with improved characteristics could be obtained by incorporating butyl (alkyl)acrylate in
6 the hydrophobic segment. One of the major advantages of polymers in accordance with the
7 present invention is the presence of the butyl chain of the butyl (alkyl)acrylate that largely
8 minimizes formation of such hydrogen bonding complexes and can prevent formation of
9 aggregates. This aids in the formation of stable supramolecular assemblies having uniform
10 size range.

11 For example, poly(ethylene oxide)-block-poly(n-butyl acrylate-co-methacrylic
12 acid) with 50:50 mole ratio of n-butyl acrylate:methacrylic acid having molecular weight
13 of about 5300 Da forms micelles of 30 nm at pH 5.0 while poly(ethylene oxide)-block-
14 poly(ethyl acrylate-co-methacrylic acid) with 50:50 mole ratio of ethyl acrylate:
15 methacrylic acid having molecular weight of about 5100 Da forms micelles of 120 nm at
16 pH 5.0, which are possibly aggregates of several micelles.

17 An oral route is the most preferred route of administration for a pharmaceutically
18 active agent. For oral delivery, the compositions can be used in the form of tablets,
19 capsules, powders, lozenges, solutions, suspensions, syrups, elixirs, and the like. The
20 pharmaceutical compositions of the present invention are administered orally. The
21 pharmaceutical compositions of the present invention can also be administered by a
22 number of other routes, including without limitation, rectally, vaginally, topically, by
23 pulmonary route, parenterally, including but not limited to intravenous, intra-arterial,

1 intramuscular, intraperitoneal or subcutaneous route.

2 The polymers in the present invention can be modified to attach targeting ligands
3 such as lectin, antibodies or fragments of antibodies, peptides, vitamins or sugar
4 molecules.

5 **EXAMPLES**

6 In all further text, figures appearing as subscript in the polymer composition indicate the
7 mole ratio of that monomer present in the hydrophobic segment of the polymer.

8 **Example 1**

9 **In vitro release of ³H-progesterone from PEO-b-poly(nBA₅₀-co-MAA₅₀)**
10 **supramolecular assemblies at different pH:**

11 Progesterone was used as a model hydrophobic drug to evaluate the effect of pH on
12 drug release from supramolecular assemblies. ³H-progesterone was loaded in the
13 supramolecular assemblies of PEO-b-poly(nBA₅₀-co-MAA₅₀) of molecular weight 5300
14 Da by film casting method. Briefly, 10 mg polymer, 1 mg progesterone and 1 μCi ³H-
15 progesterone were dissolved in a mixture of dichloromethane, ethanol and water in a
16 scintillation vial. The solvents were evaporated under reduced pressure to cast a film of
17 polymer and drug on the glass surface. The film was hydrated with water to obtain the
18 supramolecular assemblies, this solution was filtered through 2 μm filter to remove
19 precipitated drug.

20 For *in vitro* release study, the solution of progesterone loaded supramolecular
21 assemblies was filled in a dialysis bag (6000-8000 Da molecular weight cut off) and the

1 bag was put in a beaker containing 200 mL of simulated gastric fluid, pH 1.2 maintained at
2 37°C. The release medium was magnetically stirred. After 2 hours, the pH of medium was
3 adjusted to 7.2 by addition of sodium hydroxide and potassium dihydrogen phosphate.
4 During the entire release experiment, 1 mL samples of release medium were withdrawn
5 periodically to measure the radioactivity of ³H-progesterone. As a control, the release of
6 ³H-progesterone from supramolecular assemblies was also measured at pH 1.2, pH 7.2 and
7 at pH 1.2 in absence of polymer. The results of the release experiment are shown in Figure
8 1.

9 As shown in Figure 1, the progesterone is released rapidly in the absence of
10 polymer at pH 1.2, suggesting that the dialysis bag does not form a barrier for the drug
11 release. Further, the progesterone release from PEO-b-poly(nBA₅₀-co-MAA₅₀)
12 supramolecular assemblies is very rapid at pH 7.2, while slow at pH 1.2. On the other
13 hand, when the pH of the release medium is changed from 1.2 to 7.2 after 2 hours, the
14 release rate increases significantly. This is evidentiary of pH dependent dissociation of
15 supramolecular assemblies. At pH 1.2, the polymer exists in the form of supramolecular
16 assemblies due to unionized carboxyl groups and the drug is released slowly from the core
17 of supramolecular assemblies. However, when the pH is increased to 7.2, the carboxyl
18 groups become ionized resulting in the dissociation of supramolecular assemblies and the
19 drug is released rapidly.

20 To support this data, pH dependent aggregation behavior of PEO-b-poly(nBA₅₀-co-
21 MAA₅₀) was studied using dynamic light scattering. Polymer solutions (0.5 mg/mL) were
22 prepared in citrate phosphate universal buffer and the pH was adjusted between about 2.2-
23 7.0. The intensity of scattered light from these solutions at different pH was measured at

1 25°C and 90° angle and was plotted as a function of pH. The results are shown in Figure 2.

2 From Figure 2, it is evident that the scattered light intensity is negligible at pH
3 above ~5.5 while when the pH is decreased below 5.5, the intensity increases significantly
4 suggesting association of polymeric chains. This indicates that below pH 5.5 the polymer
5 is present in the form of supramolecular assemblies. The size of these supramolecular
6 assemblies is in the range of 30-100 nm depending upon the environmental pH.

7 **Example 2**

8 **Bioavailability studies of fenofibrate entrapped in supramolecular assemblies upon** 9 **oral administration to rats**

10 Fenofibrate (FNB) was used as a model poorly water-soluble hydrophobic drug to
11 evaluate the effect of drug incorporation in supramolecular assemblies on the
12 bioavailability upon oral administration to rats. In a series of experiments, FNB
13 incorporation was studied in different PEO-b-poly(EA-co-MAA) and PEO-b-poly(nBA₅₀-
14 co-MAA₅₀) polymers by emulsion and film casting methods. The FNB loading was higher
15 in PEO-b-poly(nBA₅₀-co-MAA₅₀) polymers. Therefore these polymers were used to
16 evaluate relative bioavailability of FNB loaded supramolecular assemblies in Sprague-
17 Dawley rats.

18 The study was conducted on 3 fenofibrate formulations, namely FNB
19 supramolecular assemblies, FNB standard formulation and resuspended FNB. FNB loaded
20 supramolecular assemblies were prepared from PEO-b-poly(nBA₅₀-co-MAA₅₀) of about
21 molecular weight 5300 Da by film casting method. Size of the supramolecular assemblies
22 was in the range of about 100-300 nm. FNB standard formulation was prepared by
23 suspending the powder from Lipidil Macro® (Fournier) capsule in 0.5% w/v

1 carboxymethyl cellulose sodium (CMC Na) solution to obtain uniform suspension. FNB
2 powder (Sigma) was also suspended in 0.5% w/v CMC Na solution to prepare resuspended
3 FNB formulation which acts as a negative control.

4 Rats were divided into 3 groups of 6 animals each. The rats were fasted overnight
5 and fed with standard diet throughout the study. Each formulation was administered orally
6 at a dose of 7.5 mg/kg to 6 rats from a group. Blood was removed periodically from each
7 rat, plasma was separated and stored at -80°C till further use. FNB content from the plasma
8 was determined and plotted against time, the results of which are shown in Figure 3.

9 The results show that FNB incorporated in supramolecular assemblies results in
10 highest peak plasma level, ie. 10.9 µg/mL compared to 8.4 µg/mL for standard
11 formulation. Also, t_{max} was achieved rapidly by FNB loaded supramolecular assemblies
12 compared to standard formulation. Overall, the relative bioavailability of FNB was
13 enhanced by 19% upon entrapment in supramolecular assemblies compared to standard
14 FNB formulation, and the bioavailability enhancement was 133% compared to
15 resuspended FNB powder. This enhancement in relative bioavailability is possibly due to
16 release of drug from supramolecular assemblies in the nanoscopic size range, which
17 increases the rate of dissolution of drug.

18 Example 3

19 **Formation of polyion complex micelles of PEO-*b*-P(nBA₅₀-*co*-MAA₅₀) with poly-*l*-** 20 **lysine**

21 Poly-*l*-lysine (PLL) of molecular weight 16,100 was used as a model cationic compound
22 for formation of polyion micelles with PEO-*b*-P(EA₅₀-*co*-MAA₅₀) and PEO-*b*-P(nBA₅₀-*co*-
23 MAA₅₀) copolymers with molecular weights of 5100 and 5700 Da, respectively. Polymer:

1 PLL (-/+) charge ratios (mole: mole) of 1:1 and 2:1 were used for complex formation.
 2 Stock solutions of polymer and PLL (molecular weight 16,100) having concentration of
 3 2.5 mg/mL were prepared in phosphate buffer (pH 7.4) and mixed at room temperature to
 4 obtain 1 mg/mL final polymer concentration. The solution was filtered through 0.2 μ m
 5 filter and size measurements were performed at 25°C using dynamic light scattering
 6 (DLS). The results are shown in Table 1.

7 Table 1. Size of different polymer: PLL polyion micelles

Polymer	Charge ratio (mol/mol)	Diameter (nm) mean \pm SD	% Population	Polydispersity Mean \pm SD
PEO-b-P(EA ₅₀ -co-MAA ₅₀)	1:1	1049 \pm 170	65	0.571 \pm 0.199
		35 \pm 3.2	35	
PEO-b-P(EA ₅₀ -co-MAA ₅₀)	2:1	220 \pm 5.1	100	0.448 \pm 0.024
PEO-b-P(nBA ₅₀ -co-MAA ₅₀)	1:1	31 \pm .028	100	0.058 \pm 0.012
PEO-b-P(nBA ₅₀ -co-MAA ₅₀)	2:1	32 \pm 0.2	100	0.11 \pm 0.019

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 9 The results of Table 1 show that complexation of PLL with PEO-b-P(EA₅₀-co-MAA₅₀) at
 10 different charge ratios results in formation of relatively large aggregates which could be
 11 attributed to the hydrogen bonding between poly(ethylene oxide) chain and carboxyl
 12 groups. In contrast, the complexation of PLL with PEO-b-P(nBA₅₀-co-MAA₅₀) at similar
 13 ratios results in formation of micelles having unimodal size distribution and low
 14 polydispersity indices. Similar complexes are obtained with PLL of different molecular

1 weights.

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3 Example 4

4 **Complexation of PEO-b-P(nBA₅₀-co-MAA₅₀) with verapamil hydrochloride**

5 Verapamil hydrochloride was used as a model cationic drug. Solutions of PEO-b-P(nBA₅₀-
6 co-MAA₅₀) and verapamil hydrochloride in universal buffer were mixed to obtain final
7 polymer concentration of 0.5 mg/mL and verapamil hydrochloride concentration of 0.8
8 mg/mL. The solution pH was adjusted to 6.1 and size was measured using DLS. Polyion
9 complex micelles of 38 ± 10.3 nm were obtained.

10 All patents and publications mentioned in this specification are indicative of the
11 levels of those skilled in the art to which the invention pertains. All patents and
12 publications are herein incorporated by reference to the same extent as if each individual
13 publication was specifically and individually indicated to be incorporated by reference.

14 It is to be understood that while a certain form of the invention is illustrated, it is
15 not to be limited to the specific form or arrangement of parts herein described and shown.
16 It will be apparent to those skilled in the art that various changes may be made without
17 departing from the scope of the invention and the invention is not to be considered limited
18 to what is shown and described in the specification and drawings.

19 One skilled in the art will readily appreciate that the present invention is well
20 adapted to carry out the objects and obtain the ends and advantages mentioned, as well as
21 those inherent therein. The compounds, compositions, biologically related compounds,
22 methods, procedures and techniques described herein are presently representative of the
23 preferred embodiments, are intended to be exemplary and are not intended as limitations

1 on the scope. Changes therein and other uses will occur to those skilled in the art which are
2 encompassed within the spirit of the invention and are defined by the scope of the
3 appended claims.

4 Although the invention has been described in connection with specific preferred
5 embodiments, it should be understood that the invention as claimed should not be unduly
6 limited to such specific embodiments. Indeed, various modifications of the described
7 modes for carrying out the invention which are obvious to those skilled in the art are
8 intended to be within the scope of the following claims.

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CLAIMS

2 What is claimed is:

3 1. A pharmaceutical composition comprising:

4 a diblock copolymer of poly(ethylene oxide) and poly(butyl (alkyl)acrylate-co-
5 (alkyl)acrylic acid); and at least one biologically active agent.6 2. The polymer of claim 1 wherein said poly(ethylene oxide) segment has a molecular
7 weight in the range of about 200-80,000 Da.8 3. The polymer of claim 1 wherein said poly(butyl (alkyl)acrylate-co-(alkyl)acrylic
9 acid) segment has a molecular weight in the range of about 200-80,000 Da.10 4. The polymer of claim 1 wherein said alkyl is an alkyl chain having from 0 to about
11 10 carbon atoms.12 5. The polymer of claim 1 wherein said butyl portion of the butyl (alkyl)acrylate is a
13 linear or branched chain.14 6. The polymer of claim 1 wherein said butyl (alkyl)acrylate : (alkyl)acrylic acid is in
15 a molar ratio in the range of about 5:95 to 95:5.16 7. The pharmaceutical composition of claim 1 wherein said diblock polymer is
17 poly(ethylene oxide)-block-poly(n-butyl acrylate-co-methacrylic acid) having an n-
18 butyl acrylate: methacrylic acid molar ratio of about 50:50.19 8. The pharmaceutical composition of claim 1 in the form of supramolecular
20 assemblies or micelles.21 9. The pharmaceutical composition of claim 8 wherein said supramolecular
22 assemblies or micelles are in a size range of about 5 to 1000 nanometers.

23 10. The pharmaceutical composition of claim 8 wherein said supramolecular

1 assemblies associate or dissociate reversibly in response to environmental pH
2 changes.

3 11. The pharmaceutical composition of claim 1 wherein release rate of said
4 biologically active agent increases with increase in pH.

5 12. The pharmaceutical composition of claim 8 wherein the biologically active agent is
6 a hydrophobic drug incorporated in said supramolecular assemblies by physical or
7 chemical methods.

8 13. The pharmaceutical composition of claim 12 wherein the hydrophobic drug is
9 fenofibrate.

10 14. The pharmaceutical composition of claim 8 wherein the biologically active agent is
11 a cation or polycation.

12 15. The pharmaceutical composition of claim 14 wherein said polycation is a peptide
13 or protein bearing cationic residues.

14 16. The pharmaceutical composition of claim 14 wherein the cation or polycation
15 interacts electrostatically with said (alkyl)acrylic acid units.

16 17. The pharmaceutical composition of claim 14 wherein the cation is verapamil
17 hydrochloride.

18 18. The pharmaceutical composition of claim 8 wherein the biologically active agent
19 forms metal coordination complexes with said (alkyl)acrylic acid units.

20 19. The pharmaceutical composition of claim 18 wherein the biologically active agent
21 is cisplatin.

22 20. The pharmaceutical composition of claim 18 wherein the biologically active agent
23 is carboplatin.

1 21. The pharmaceutical composition of claim 12 wherein incorporation of a
2 hydrophobic drug in the supramolecular assemblies enhances the bioavailability of
3 the hydrophobic drug upon oral administration.

4 22. The pharmaceutical composition of claim 8 which is administered by oral,
5 intravenous, intra-arterial, subcutaneous, intramuscular, intraperitoneal, rectal,
6 vaginal or topical route.

7 23. The pharmaceutical composition of claim 8 having a targeting ligand on a surface
8 thereof.

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FIGURE 1

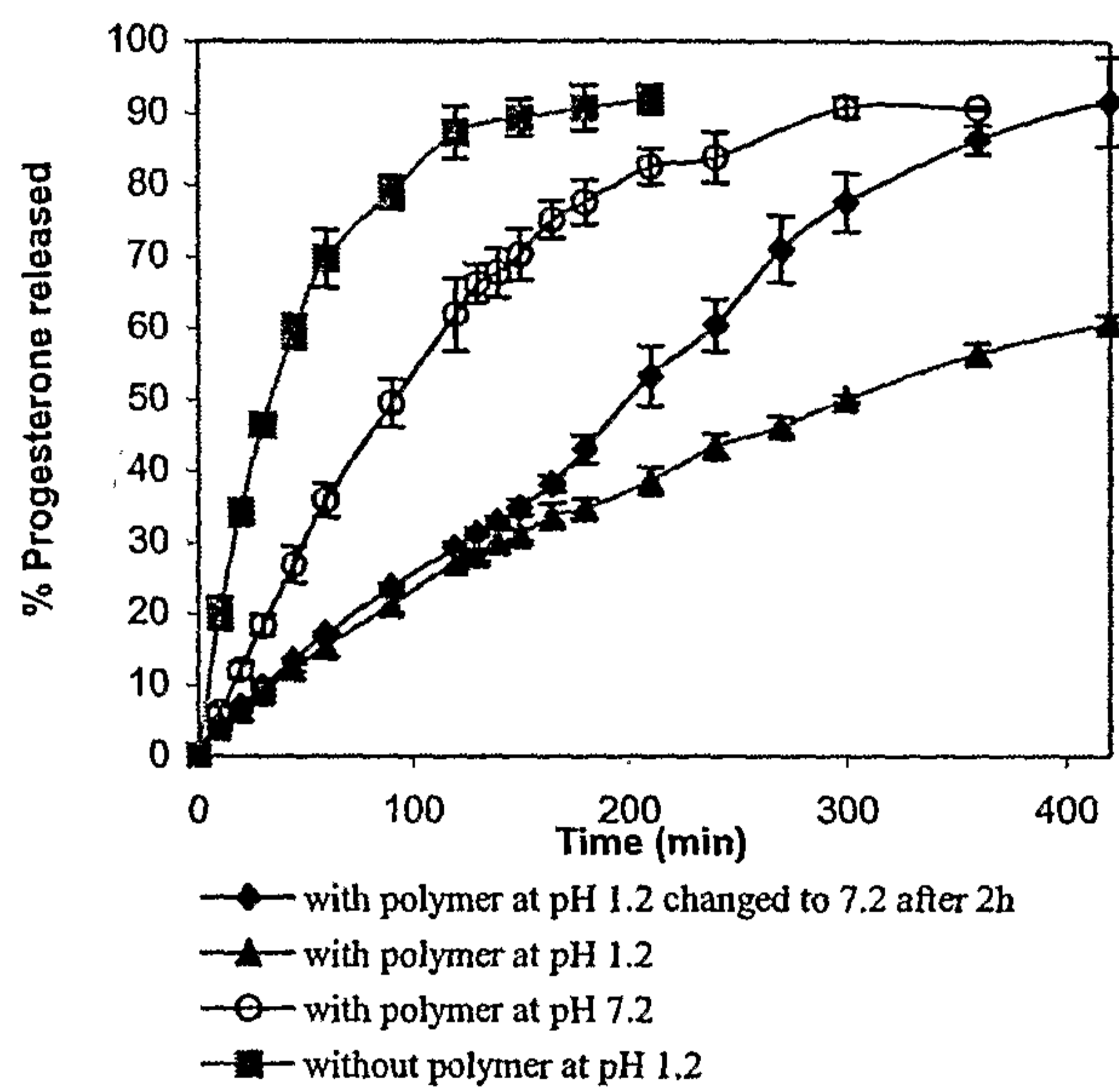
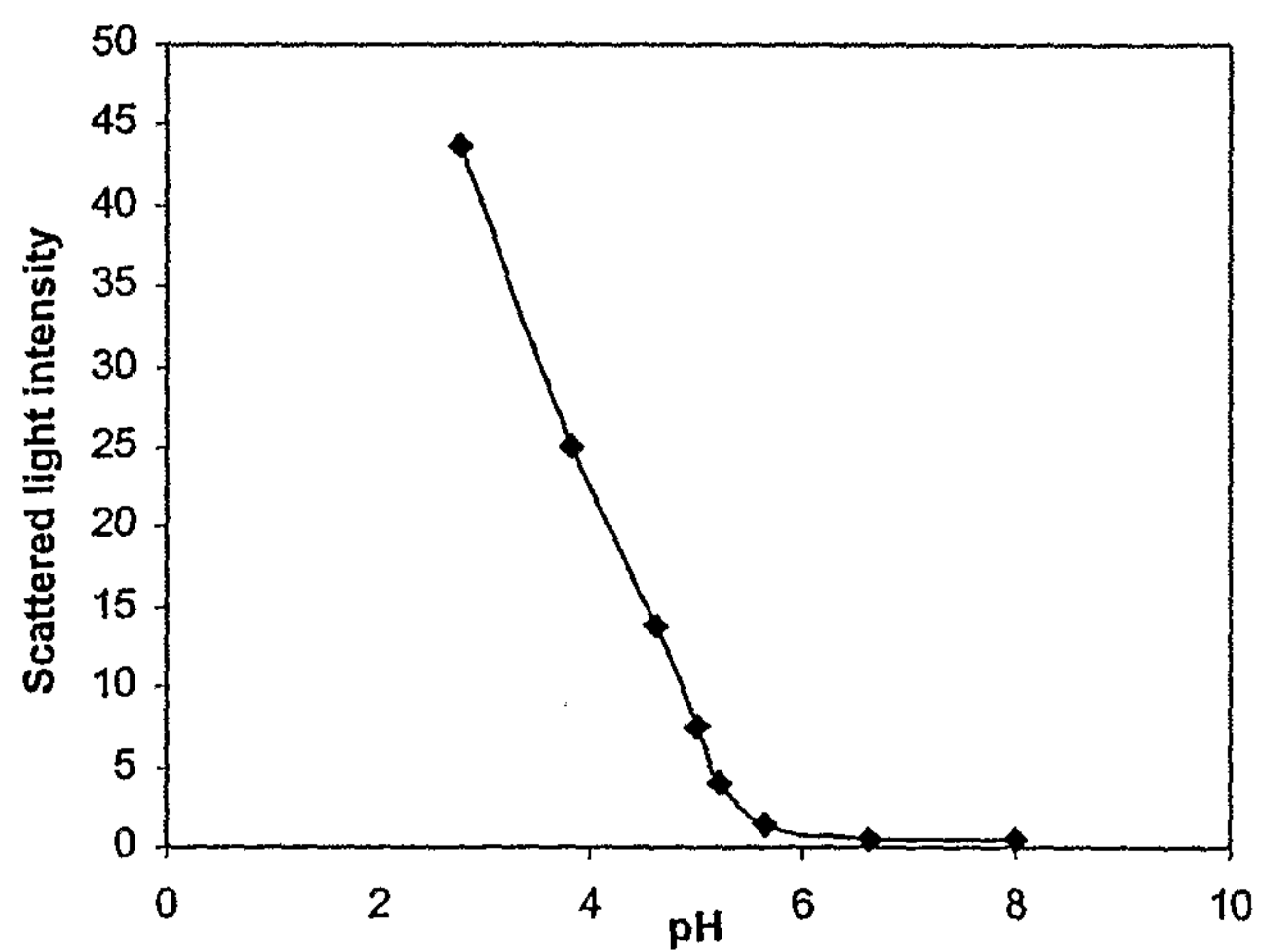
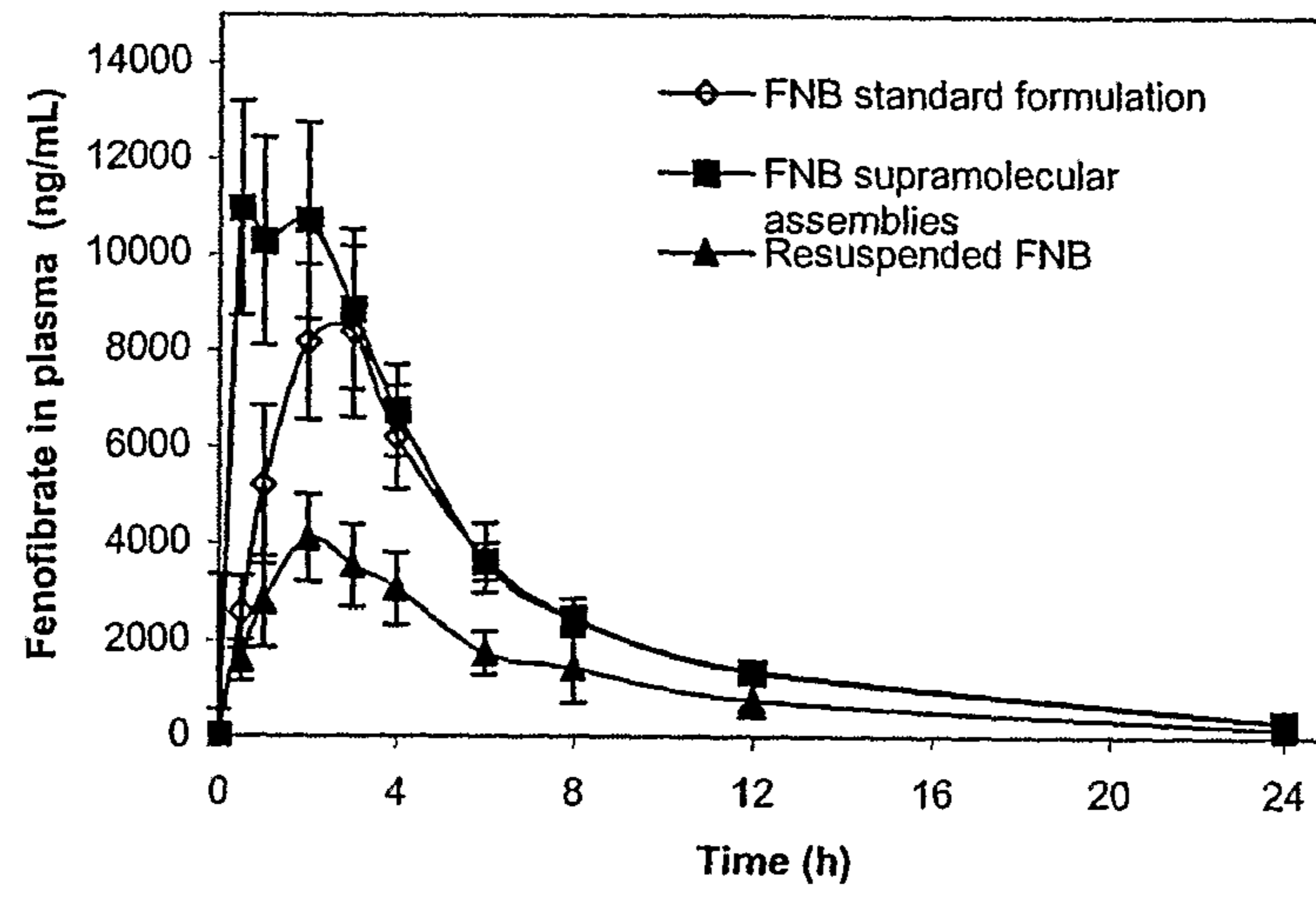


FIGURE 2

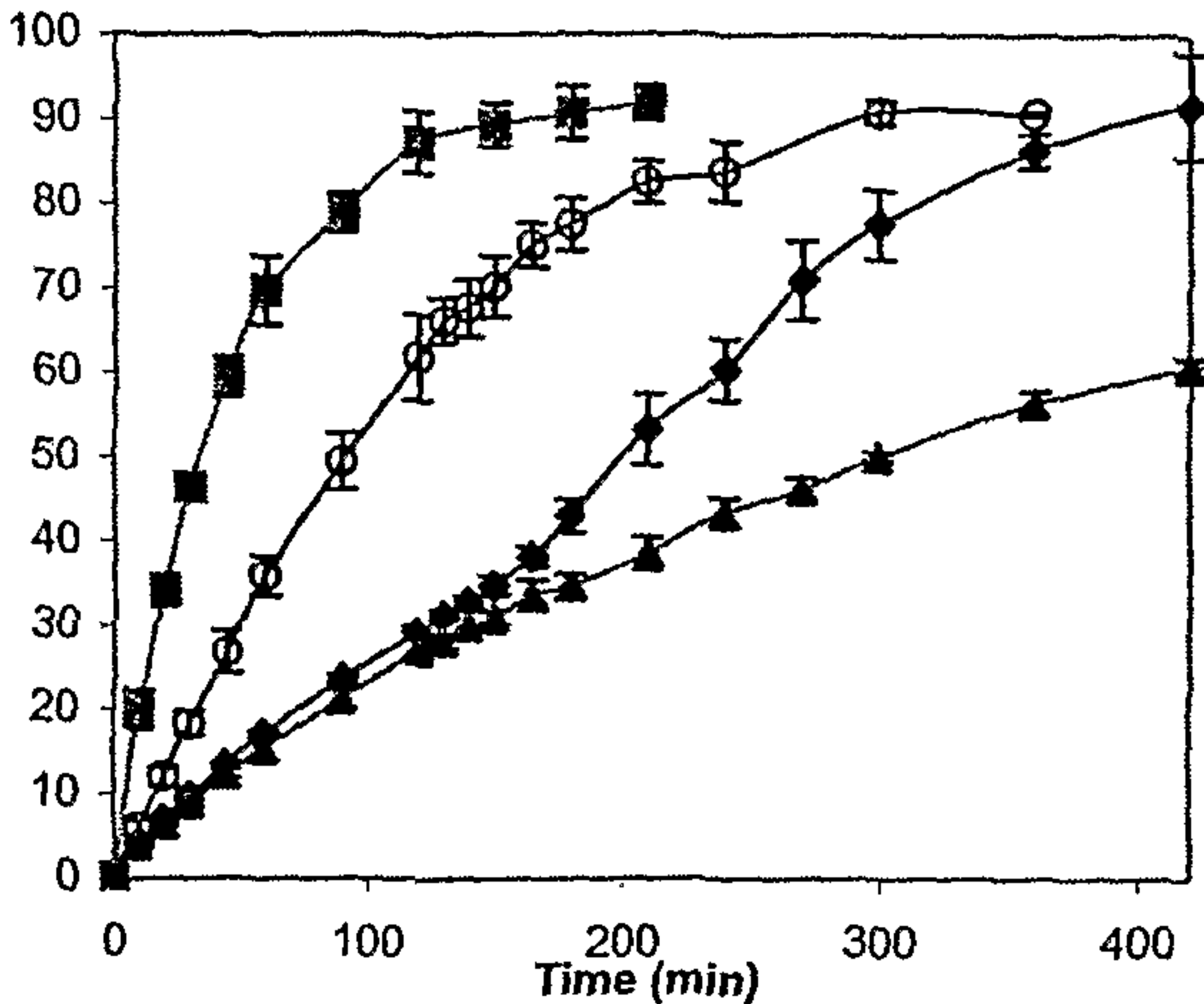


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FIGURE 3



% Progesterone released



- ◆— with polymer at pH 1.2 changed to 7.2 after 2h
- ▲— with polymer at pH 1.2
- with polymer at pH 7.2
- without polymer at pH 1.2