



(86) Date de dépôt PCT/PCT Filing Date: 2004/11/08  
(87) Date publication PCT/PCT Publication Date: 2005/06/09  
(85) Entrée phase nationale/National Entry: 2006/05/01  
(86) N° demande PCT/PCT Application No.: EP 2004/012748  
(87) N° publication PCT/PCT Publication No.: 2005/051997  
(30) Priorité/Priority: 2003/11/27 (EP03257478.2)

(51) Cl.Int./Int.Cl. *C07K 19/00* (2006.01)  
(71) Demandeur/Applicant:  
UNILEVER PLC, GB  
(72) Inventeurs/Inventors:  
BUSHELL-WILLIAMS, DONNA, GB;  
PARRY, NEIL JAMES, GB;  
WINDUST, JOHN HERBERT C., GB;  
ZUIDAM, NICOLAAS JAN, NL  
(74) Agent: BERESKIN & PARR

(54) Titre : PROTEINES DE FUSION ET COMPOSITIONS DETERGENTES LES CONTENANT  
(54) Title: FUSION PROTEINS AND DETERGENT COMPOSITIONS COMPRISING THEM

(57) **Abrégé/Abstract:**

There is provided a fusion protein comprising a Carbohydrate Binding Domain and a domain having a high binding affinity for a melamine-type polymer. Also provided is a detergent composition comprising one or more surfactants and a fusion protein according to the invention and micro-particles comprising a melamine-type polymer, which contain a benefit agent selected from the group consisting of a fabric softening agents, fragrances, perfumes, polymeric lubricants, photoprotective agents, dye fixative agents, antioxidants, insecticides, soil repelling agents or a soil release agents. The fusion protein is advantageously used in a detergent composition to deliver benefit agents to a fabric.



## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
9 June 2005 (09.06.2005)

PCT

(10) International Publication Number  
**WO 2005/051997 A3**

(51) International Patent Classification<sup>7</sup>: **C07K 19/00**

(21) International Application Number:  
PCT/EP2004/012748

(22) International Filing Date:  
8 November 2004 (08.11.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
03257478.2 27 November 2003 (27.11.2003) EP

(71) Applicant (for AL, AM, AT, AZ, BA, BE, BF, BG, BJ, BR, BY, CF, CG, CH, CI, CM, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, FR, GA, GE, GN, GQ, GR, GW, HR, HU, ID, IS, IT, JP, KG, KP, KR, KZ, LR, LT, LU, LV, MA, MC, MD, MG, MK, ML, MR, MX, MZ, NA, NE, NI, NL, NO, PH, PL, PT, RO, RU, SE, SI, SK, SN, SY, TD, TG, TJ, TM, TN, TR, UA, UZ, VN, YU only): **UNILEVER N.V.** [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).

(71) Applicant (for AE, AG, AU, BB, BW, BZ, CA, CY, EG, GB, GD, GH, GM, IE, IL, KE, LC, LK, LS, MN, MW, NZ, OM, PG, SC, SD, SG, SL, SZ, TT, TZ, UG, VC, ZA, ZM, ZW only): **UNILEVER PLC** [GB/GB]; UNILEVER HOUSE, Blackfriars, London Greater London EC4P 4BQ (GB).

(71) Applicant (for IN only): **HINDUSTAN LEVER LIMITED** [IN/IN]; HINDUSTAN LEVER HOUSE, 165/166 Backbay Reclamation, Maharashtra, Mumbai 400 020 (IN).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BUSHELL-WILLIAMS, Donna** [GB/GB]; UNILEVER R & D COLWORTH, Sharnbrook, Bedford Bedfordshire MK44 1LQ (GB). **PARRY, Neil, James** [GB/GB]; UNILEVER R & D COLWORTH, Sharnbrook, Bedford Bedfordshire MK44

1LQ (GB). **WINDUST, John, Herbert, C.** [GB/GB]; UNILEVER R & D COLWORTH, Sharnbrook, Bedford Bedfordshire MK44 1LQ (GB). **ZUIDAM, Nicolaas, Jan** [NL/NL]; UNILEVER R & D VLAARDINGEN, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL).

(74) Agent: **KAN, Jacob, H.**; UNILEVER Intellectual Property Group, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:  
1 September 2005

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.

(54) Title: FUSION PROTEINS AND DETERGENT COMPOSITIONS COMPRISING THEM

(57) Abstract: There is provided a fusion protein comprising a Carbohydrate Binding Domain and a domain having a high binding affinity for a melamine-type polymer. Also provided is a detergent composition comprising one or more surfactants and a fusion protein according to the invention and micro-particles comprising a melamine-type polymer, which contain a benefit agent selected from the group consisting of a fabric softening agents, fragrances, perfumes, polymeric lubricants, photoprotective agents, dye fixative agents, antioxidants, insecticides, soil repelling agents or a soil release agents. The fusion protein is advantageously used in a detergent composition to deliver benefit agents to a fabric.

WO 2005/051997 A3

**FUSION PROTEINS AND DETERGENT COMPOSITIONS COMPRISING THEM****TECHNICAL FIELD**

5 The present invention generally relates fusion proteins and detergent compositions comprising them. More in particular, it relates to detergent compositions comprising fusion proteins which are useful for delivering a benefit agent to a fabric.

10

**BACKGROUND AND PRIOR ART**

In a laundry cleaning process it is sometimes desirable to deliver benefit agents onto the fabric. Such a benefit agent may for example be a fabric softening agent, a perfume, a  
15 polymeric lubricant, a photoprotective agent, a latex, a resin, a dye fixative agent, an encapsulated material, an antioxidant, an insecticide, a soil repelling agent or a soil release agent.

20 From WO-A-98/00500 (Unilever) it is known to deliver a benefit agent onto fabric by means of peptide or protein Deposition Aid having a high affinity for fabric. The benefit agent is attached or adsorbed to a peptide or protein Deposition Aid having a high affinity for fabric.

25 Preferably, the deposition aid is the cellulose binding domain of a cellulase enzyme. The compositions are said to effectively deposit the benefit agent onto the fabric during the wash cycle.

30 In order to overcome certain drawbacks and limitations of WO-A-98/00500, it has been further proposed in WO-A-01/46357 (Unilever) to use fusion proteins to deposit the benefit agents onto the fabric. The fusion proteins comprise a cellulose binding domain for binding to the fabric and a  
35 domain having a high binding affinity for the benefit agent.



However, the stability of detergent compositions disclosed in WO-A-01/46357, may under certain circumstances, leave to be desired. It was found that many of the available technologies to encapsulate the payload of benefit agent may not give sufficiently stable encapsulates when they are formulated into a detergent composition. Subsequently, the benefit agent may leak out of the capsule during processing of the formulation or upon storage.

10 It is an object of the present invention to provide an alternative or improved detergent composition, which is capable of delivering a benefit agent to a fabric during a washing or rinsing process.

15 Importantly, the use of chemically defined capsules is key to maintain the integrity of capsules during the formulation and storage stages of a detergent formulation. However, because these chemical species are very simple in structure, the ability to obtain a protein-binding molecule to specifically recognise these cross-linked repeating haptens cannot be readily envisaged.

Surprisingly, we have now found that these and other objects of the invention may be achieved by the fusion protein of the present invention, which is characterised in that it comprises a Carbohydrate Binding Domain, preferably a Cellulose Binding domain, and a domain having a high binding affinity for a melamine-type polymer. We have found that, by means of such fusion proteins, it is possible to enhance the delivery and retention of perfume encapsulated in melamine / formaldehyde based polymer capsules to fabric in a main wash formulation.

This fusion molecule is bi-functional in its binding ability, whereby the Carbohydrate Binding Domain region binds to carbohydrate such as cellulosic fabric materials

and the second domain binds to a melamine-type polymer. In this way, melamine capsules containing benefit agents may be targeted to the fabric to deliver the benefit agent onto the fabric.

5

Furthermore, the same capsule form can be used to deliver a whole range of benefit agents, therefore the same protein binding molecules may be used to delivery a whole range of benefit agents utilising a generic capsule approach.

10

#### DEFINITION OF THE INVENTION

According to a first aspect of the invention, there is provided a fusion protein comprising a Carbohydrate Binding Domain and a domain having a high binding affinity for a melamine-type polymer.

15

According to a second aspect, there is provided a detergent composition comprising such fusion protein and micro-particles containing a benefit agent.

20

According to a third aspect, there is provided a process for delivering a benefit agent to a fabric by treating said fabric with a composition comprising said fusion protein and a micro-particles containing a benefit agent.

25

#### DETAILED DESCRIPTION OF THE INVENTION

##### 1.1 The Carbohydrate Binding Domain

In its first aspect, the invention relates to a fusion protein comprising a Carbohydrate Binding Domain (CBD) and a domain having a high binding affinity for a melamine-type polymer. A Carbohydrate Binding Domain is a polypeptide that has a high affinity for or binds to water-soluble or water-insoluble forms of carbohydrate polymers such as cellulose and chitin, including their crystalline forms. CBDs are

30  
35



sometimes also referred to as polysaccharide binding domains (PBDs).

Preferred examples of Carbohydrate Binding Domains are  
5 Cellulose Binding Domains. The term Carbohydrate Binding Domain is broader and also includes protein sequences such as starch binding domains, mannose binding domains, xylan binding domains and chitin binding domains. Henrissat B. and Davies G.J. (*Plant Physiology* (2000) 124(4):1515-1519)  
10 describe many examples of such Carbohydrate Binding Domains. The term Carbohydrate Binding Domain (or CBD) will be collectively used hereinafter for any of such binding domains which can be used as part of the fusion protein according to the present invention, unless indicated  
15 otherwise.

A preferred Carbohydrate Binding Domain is the Cellulose Binding Domain, which will be used hereinafter as a typical example of the Carbohydrate Binding Domain part of the  
20 fusion protein according to the present invention. Thus, the abbreviation CBD is used hereinafter to indicate Carbohydrate Binding Domains in general, whereas a Cellulose Binding Domain is a typical and preferred example of the broader definition of Carbohydrate Binding Domains.

25

Carbohydrate Binding Domains are found as integral parts of large protein complexes consisting of two or more different polypeptides, for example in hydrolytic enzymes (hydrolases) which typically are composed of a catalytic domain  
30 containing the active site for substrate hydrolysis, and a Carbohydrate Binding Domain such as a Cellulose Binding Domain for binding to the insoluble matrix. Such enzymes may comprise more than one catalytic domain and one, two or three CBDs and optionally one or more polypeptide regions  
35 linking the CBD(s) with the catalytic domain(s), the latter regions usually being denoted a "linker". Examples of

hydrolytic enzymes comprising a CBD are cellulases, xylanases, mannanases, arabinofuranosidases, acetyl esterases and chitinases. CBDs have also been found in algae, e.g. the red alga *Porphyra purpurea* as a non-  
5 hydrolytic polysaccharide binding protein, see Peter Tomme et al., "Cellulose Binding Domains: Classification and Properties" in "Enzymatic Degradation of Insoluble Carbohydrates", John N. Saddler and Michael H. Penner (Eds.), ACS Symposium Series, No. 618, 1996. However, most  
10 of the known CBDs are from cellulases and xylanases.

In this context, the term "Cellulose Binding Domain" is intended to be understood as defined by Tomme et al., op. cit. This definition classifies more than 120 Cellulose  
15 Binding Domains into 10 families (I-X) which may have different functions or roles in connection with the mechanism of substrate binding. However, it is anticipated that new family representatives and additional CBD families will appear in the future.

20

A Carbohydrate Binding Domain may be exemplified by a binding domain recognising cellulose. Such a Cellulose Binding Domain is a part of many cellulolytic enzymes and can be obtained therefrom. Cellulose Binding Domains are  
25 also obtainable from xylanase and other hemicellulase degrading enzymes. Preferably, the Cellulose Binding Domain is obtainable from a fungal enzyme origin such as Humicola, Trichoderma, Thermomonospora, Phanerochaete, Aspergillus, Meripilus or from a bacterial enzyme origin such as  
30 Bacillus, Clostridium, Streptomyces, Cellulomonas and Pseudomonas.

In the fusion protein according to the invention, the Carbohydrate Binding Domain is fused, using conventional  
35 rDNA techniques, to a second domain having a high binding affinity for a melamine-type polymer. Preferably, the



Carbohydrate Binding Domain is connected to the domain having a high binding affinity for the melamine-type polymer by means of a linker consisting of about 0-20, preferably about 2-15, more preferably of 2-5 amino acid residues.

5

The second domain having a high binding affinity for the melamine-type polymer may, for instance, be an antibody or an antibody fragment. Especially preferred are heavy chain antibodies such as found in Camelidae.

10

Llama antibodies have been shown to be able to bind to both proteins and dye haptens. Published work on their structure and functionality states that raising single chain antibodies against haptens turned out to be difficult if not impossible, especially with hydrophobic ones (Journal of Molecular Biology 2001 311, 123-129). It was shown that it was possible to generate binders to a variety of dye haptens. These characteristically contained a number of benzene rings and sulphonated side chains.

20

The present inventors have now isolated 3 antibody binding domains from a library size of  $10^{11}$  that are capable of binding to melamine particles. It was surprising that binders to melamine were found, considering that melamine represents a repeating unit of C<sub>3</sub>N<sub>6</sub>H<sub>6</sub> [1,3,5 triazine 2,4,6 triamine].

25

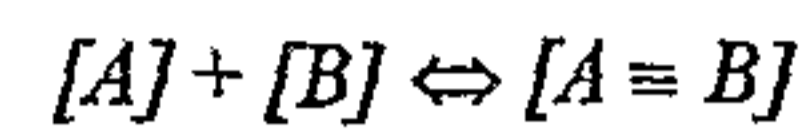
The fusion protein according to the invention may comprise more than two recognition domains. It is for example possible to produce a CBD fusion protein with more than one antibody domain, in which the antibody domains may bind to the same or bind to different antigens. Conversely, it is also possible in the CBD antibody fusion format to produce a molecule with one antibody domain with more than one CBD, whereby the CBD's incorporated may be identical sequences or from more than one source, or modified varieties thereof.

30

35



Generally speaking, the degree of binding of a molecule A to another molecule B can be generally expressed by the chemical equilibrium constant  $K_d$  resulting from the following reaction:



The chemical equilibrium constant  $K_d$  is then given by:

$$K_d = \frac{[A][B]}{[A \equiv B]}$$

Whether the binding of a molecule to the fabric / ligand is specific or not can be judged from the difference between the binding ( $K_d$  value) of the molecule to one type of fabric / ligand, versus the binding to another type of fabric / ligand material. For applications in laundry, said ligand material will form part of or be associated with a benefit agent. In this aspect of the invention, the CBD region of the fusion protein binds to the fabric and the high affinity domain region binds to the melamine-type polymer agent.

Alternatively, this approach can be reversed whereby the fabric material, or a ligand bound to the fabric is targeted by the high affinity domain of the fusion protein and the cellulose binding domain region binds to a cellulosic based or cellulosic containing benefit agent.

However, it will usually be more convenient to measure  $K_d$  values and differences in  $K_d$  values on other materials such as a polystyrene microtitre plate or a specialised surface in an analytical biosensor. The difference between the two binding constants should be minimally 10, preferably more than 100, and more preferably, more than 1000. Typically, the reagent should bind to the ligand / fabric, with a  $K_d$  lower than  $10^{-4}$  M, preferably lower than  $10^{-6}$  M and could be  $10^{-10}$  M or even less. Higher binding affinities ( $K_d$  of less

than  $10^{-5}$  M) and/or a larger difference between the one type of ligand / fabric and another type of ligand / fabric (or background) binding would increase the deposition of the melamine-type polymer in the micro-particles containing the benefit agent. Also, the weight efficiency of the reagent in the total rinse composition would be increased and smaller amounts of the reagent would be required.

Several classes of reagent or molecules can be envisaged which deliver the capability of specific binding to fabrics / ligands, to which one would like to deliver the benefit agent. In the following we will give a number of examples of molecules having such capabilities, without pretending to be exhaustive.

15

#### 1.2.1. Antibodies.

Antibodies are specific binding proteins. Their function in nature is to protect against disease by recognising (and binding) foreign bodies, such as viruses or bacteria, but not self-cells. Furthermore, methods are well-known in the art to generate antibodies that are specific for almost any protein, organic molecule, or cell surface, that is likely to be encountered. This binding specificity has been exploited in the Biotechnology industry, principally for medical diagnostics. For example, many home-based pregnancy test kits comprise an antibody that specifically binds to the pregnancy marker hormone, human chorionic gonadotropin (hCG), but not to other hormones present in urine.

More recently, the use of antibodies in laundry products has been described. In particular, Unilever has described the use of stain-specific antibodies to target bleaching enzymes exclusively to stains but not to dyes - thus achieving efficient stain removal without damaging surrounding fabric (WO-A-98/56885).

35



Antibodies are well known examples of molecules that are capable of binding specifically to compounds against which they were raised. Antibodies can be derived from several sources. From mice, monoclonal antibodies can be obtained  
5 which possess very high binding affinities. From such antibodies, Fab, Fv or scFv fragments, can be prepared which have retained their binding properties. Such antibodies or fragments can be produced through recombinant DNA technology by microbial fermentation. Well known production hosts for  
10 antibodies and their fragments are yeast, moulds or bacteria.

A class of antibodies of particular interest is formed by the Heavy Chain antibodies as found in Camelidae, like the  
15 camel or the llama. The binding domains of these antibodies consist of a single polypeptide fragment, namely the variable region of the heavy chain polypeptide (HC-V). In contrast, in the classic antibodies (murine, human, etc.), the binding domain consists of two polypeptide chains (the  
20 variable regions of the heavy chain ( $V_h$ ) and the light chain ( $V_l$ )). Procedures to obtain heavy chain immunoglobulins from Camelidae, or (functionalized) fragments thereof, have been described in WO-A-94/04678 (Casterman and Hamers) and WO-A-94/25591 (Unilever and Free University of Brussels).

25

Alternatively, binding domains can be obtained from the  $V_h$  fragments of classical antibodies by a procedure termed "camelization". Hereby the classical  $V_h$  fragment is transformed, by substitution of a number of amino acids,  
30 into a HC-V-like fragment, whereby its binding properties are retained. This procedure has been described by Riechmann et al. in a number of publications (J. Mol. Biol. (1996) **259**, 957-969; Protein. Eng. (1996) **9**, 531-537, Bio/Technology (1995) **13**, 475-479). Also HC-V fragments can  
35 be produced through recombinant DNA technology in a number

of microbial hosts (bacterial, yeast, mould), as described in WO-A-94/29457 (Unilever).

Methods for producing fusion proteins that comprise an enzyme and an antibody or that comprise an enzyme and an antibody fragment are already known in the art. One approach is described by Neuberger and Rabbits (EP-A-194 276). A method for producing a fusion protein comprising an enzyme and an antibody fragment that was derived from an antibody originating in *Camelidae* is described in WO-A-94/25591. A method for producing bispecific antibody fragments is described by Holliger et al. (1993) PNAS **90**, 6444-6448.

A particularly attractive feature of antibody binding behaviour is their reported ability to bind to a "family" of structurally related molecules. For example, in Gani et al. (J. Steroid Biochem. Molec. Biol. **48**, 277-282) an antibody is described that was raised against progesterone but also binds to the structurally-related steroids, pregnanedione, pregnanolone and 6-hydroxy-progesterone. Therefore, using the same approach, antibodies could be isolated that bind to a whole "family" of stain chromophores (such as the polyphenols, porphyrins, or caretenoids as described below). A broad action antibody such as this could be used to treat several different stains when coupled to a bleaching enzyme.

### 1.2.2. Peptides.

Peptides usually have lower binding affinities to the substances of interest than antibodies. Nevertheless, the binding properties of carefully selected or designed peptides can be sufficient to deliver the desired selectivity in an oxidation process. A peptide which is capable of binding selectively to a fabric / ligand to which one would like to deliver a benefit agent, can for instance be obtained from a protein which is known to bind to that specific fabric / ligand. An example of such a peptide would



be a binding region extracted from an antibody raised against that fabric / ligand. A suitable peptide could be analogous to the active centre of a protein analogous to a non-catalytic binding domain of a protein, e.g. a receptor.

5

Alternatively, peptides that bind to such substance can be obtained by the use of peptide combinatorial libraries. Such a library may contain up to  $10^{10}$  peptides, from which the peptide with the desired binding properties can be isolated.

10 (R.A. Houghten, Trends in Genetics, Vol 9, no 6, 235-239). Several embodiments have been described for this procedure (J. Scott et al., Science (1990) **249**, 386-390; Fodor et al., Science (1991) **251**, 767-773; K. Lam et al., Nature (1991) **354**, 82-84; R.A. Houghten et al., Nature (1991) **354**, 84-86).

15

Suitable peptides can be produced by organic synthesis, using for example the Merrifield procedure (Merrifield (1963) J.Am.Chem.Soc. **85**, 2149-2154). Alternatively, the peptides can be produced by recombinant DNA technology in  
20 microbial hosts (yeast, moulds, bacteria) (K.N. Faber et al. (1996) Appl. Microbiol. Biotechnol. **45**, 72-79).

### 1.2.3. Peptidomimics.

In order to improve the stability and/or binding properties  
25 of a peptide, the molecule can be modified by the incorporation of non-natural amino acids and/or non-natural chemical linkages between the amino acids. Such molecules are called peptidomimics (H.U. Saragovi et al. (1991) Bio/Technology **10**, 773-778; S. Chen et al. (1992) Proc.Natl.Acad. Sci. USA **89**, 5872-5876). The production of  
30 such compounds is restricted to chemical synthesis.

### 1.2.4. Other organic molecules.

It can be readily envisaged that other molecular structures,  
35 which need not be related to proteins, peptides or derivatives thereof, can be found which bind selectively to

fabrics / ligands to which one would like to deliver a benefit agent. For example, certain polymeric RNA molecules which have been shown to bind small synthetic dye molecules (A. Ellington et al. (1990) Nature **346**, 818-822). Such  
5 binding compounds can be obtained by the combinatorial approach, as described for peptides (L.B. McGown et al. (1995), Analytical Chemistry, 663A-668A).

This approach can also be applied for purely organic  
10 compounds which are not polymeric. Combinatorial procedures for synthesis and selection for the desired binding properties have been described for such compounds (Weber et al. (1995) Angew.Chem.Int.Ed.Engl. **34**, 2280-2282; G. Lowe (1995), Chemical Society Reviews **24**, 309-317; L.A. Thompson  
15 et al. (1996) Chem. Rev. **96**, 550-600). Once suitable binding compounds have been identified, they can be produced on a larger scale by means of organic synthesis.

A further embodiment of the present invention would be for  
20 the domain with a high binding affinity to the melamine-type polymer to be a bi-specific reagent. Such a reagent could fulfil the requirement of accumulating the benefit agent on the fabric either by supplying said reagent together with the benefit agent as a pre-formed non-covalent complex or by  
25 supplying the two separately and allowing them to self-assemble either in the wash liquor or on the fabric.

## 2. The melamine-type polymer

The fusion proteins according to the invention comprises a  
30 part having a high binding affinity for melamine-based polymers, such that they can bind to micro-capsules made of or containing such chemicals and polymers. Utilising and producing melamine capsules is well known in the art, see for instance WO-A-01/51197, WO-A-01/49817, US-A-6 248 703.  
35 They contain and are characterised by the repeating unit of C<sub>3</sub>N<sub>6</sub>H<sub>6</sub> [1,3,5 triazine 2,4,6 triamine].



These melamine polymers are advantageously used in the manufacture of micro-capsules, preferably having a particle size of between 0.1 and 100 micrometer, more preferably of between 50 And 10 micrometer. Such micro-capsules are well known and have been described in US-A-2003078043, JP-A-10139817, WO-A-03/035245, US-A-6 080 418. These melamine-polymer containing micro-capsules contain the benefit agents which is to be deposited onto the fabric. Many processes for microencapsulation are known. These include methods for capsule formation such as described in US-A-2 730 456, US-A-2 800 457 and US-A-2 800 458. Other useful methods for microcapsule manufacture are described in: US-A-4 001 140, US-A-4 081 376 and US-A-4 089 802 describing a reaction between urea and formaldehyde; US-A-4 100 103 describing reaction between melamine and formaldehyde; GB-A-2 062 570 describing a process for producing microcapsules having walls produced by polymerization of melamine and formaldehyde in the presence of a styrenesulfonic acid. Micro-encapsulation is also taught in US-A-2 730 457 and US-A-4 197 346. Processes for forming microcapsules from urea-formaldehyde resin and/or melamine formaldehyde resin are disclosed in US-A-4 001 140, US-A-4 081 376, US-A-4 089 802, US-A-4 100 103, US-A-4 105 823, US-A-4 444 699. Alkyl acrylate/acrylic acid copolymer capsules are taught in US-A-4 552 811.

### 3. The detergent composition

In the detergent composition according to the invention, the fusion protein can be used to deposit a benefit agent onto the fabric by means of the Carbohydrate Binding Domain.

In a further embodiment, the detergent compositions of the invention comprise micro-particles comprising melamine-type polymers, sensitised with CBD-antibody fusions, and configured such that the micro-particles are loaded with the

benefit agent and the antibody domain of the fusion protein has a high affinity or specificity for a substance (or "marker molecule") typically found on some regions of fabrics but not on others. Examples of such marker molecules include bleach-damaged dyes and microbes known to be associated with malodour. The antibody domain targets the benefit agent to its intended site of action and binds it there. For example, Microbe-specific antibody based fusions may target fragrance-containing particles to the regions of malodour. Thus, a more efficient use of expensive ingredients is achieved. Alternatively, antibody based fusions specific for bleach-damaged dyes can target dyed particles to faded regions, thus replenishing the colour lost in the main wash cycle.

15

The CBD antibody fusion protein binds to the fabric via the CBD region, thereby allowing the antibody domain to bind to corresponding antigens / ligands that comprise or form part of the benefit agent. The fusion protein can be dosed in conjunction with the benefit agent or can be added to the fabric prior to the said fabric coming into contact with the benefit agent.

20

A further aspect of the invention is a process for delivering a benefit agent to a fabric by treating said fabric with a composition comprising a fusion protein according to the invention and micro-capsules comprising a benefit agent selected from the group consisting of softening agents, finishing agents/ protective agents, fragrances (perfumes) and bleaches.

30

Examples of softening agents are clays, cationic surfactants or silicon compounds. Examples of finishing agents/ protective agents are polymeric lubricants, soil repelling agents, soil release agents, photo-protective agents (sunscreens), anti-static agents, dye-fixing agents, anti-

35



bacterial agents and anti-fungal agents. The fragrances or perfumes inside the micro-capsules may be further encapsulated, e.g. in latex micro-capsules or they may be gelatine based coacervates.

5

Suitable examples of bleaches are photobleaches. Examples of photobleaches are given in EP-A-379 312 (British Petroleum), which discloses a water-insoluble photobleach derived from anionically substituted porphine, and in EP-A-035 470 (Ciba Geigy), which discloses a textile treatment composition comprising a photobleaching component.

10

Another advantage of the present invention is that it is possible to target some benefit molecules to particular regions of fabric. For example, dyes can be targeted to colour-bleached regions to replenish dye lost in the main wash or fragrance can be targeted to regions where it is most needed, in particular to those regions where microbes associated with malodour are present, such as the "underarm" regions.

15

20

#### 4. The fabrics

For laundry detergent applications, several classes of natural or man-made fabrics can be envisaged, in particular cotton. In the embodiment of the invention whereby the antibody region of the fusion protein targets the fabric, such macromolecular compounds have the advantage that they can have a more immunogenic nature, i.e. that it is easier to raise antibodies against them. Furthermore, they are more accessible at the surface of the fabric than for instance coloured substances in stains, which generally have a low molecular weight.

25

30

An important embodiment of the invention is to use binding domains (as described above) that bind to several different types of fabrics. This would have the advantage of enabling

35

a benefit agent to be deposited to several different types of fabric using the CBD-antibody fusion molecule.

5. The Detergent Composition.

5 The fusion proteins of the invention can be used in a detergent composition that is specifically suited for the purpose, and this constitutes a second aspect of the invention. When formulating a detergent composition, it is important to ensure that the other ingredients of the  
10 product are compatible with the activity of the fusion protein. WO-A-98/07820 (P&G) discloses *inter alia* rinse treatment compositions containing antibodies directed at cellulase and standard softener actives such as DEQA. The detergent product according to the present invention  
15 preferably contains no softener or low levels of softener active (e.g. HEQ).

To that extent, the detergent composition comprises one or more benefit agents and optionally other conventional  
20 detergent ingredients. The invention in its second aspect provides a detergent composition which comprises from 0.1 - 50 % by weight, based on the total composition, of one or more surfactants. This surfactant system may in turn  
25 comprise 0 - 95 % by weight of one or more anionic surfactants and 5 - 100 % by weight of one or more nonionic surfactants. The surfactant system may additionally contain amphoteric or zwitterionic detergent compounds, but this is not normally desired owing to their relatively high cost. It may be advantageous to also include cationic surfactants  
30 into the composition. Examples of suitable cationic surfactants are given in WO-A-97/03160 and WO-A-98/17767 (Procter&Gamble).

In general, the nonionic and anionic surfactants of the  
35 surfactant system may be chosen from the surfactants described "Surface Active Agents" Vol. 1, by Schwartz &



Perry, Interscience 1949, Vol. 2 by Schwartz, Perry & Berch, Interscience 1958, in the current edition of "McCutcheon's Emulsifiers and Detergents" published by Manufacturing Confectioners Company or in "Tenside-Taschenbuch", H. Stache, 2nd Edn., Carl Hauser Verlag, 1981.

Suitable nonionic detergent compounds which may be used include, in particular, the reaction products of compounds having a hydrophobic group and a reactive hydrogen atom, for example, aliphatic alcohols, acids, amides or alkyl phenols with alkylene oxides, especially ethylene oxide either alone or with propylene oxide. Specific nonionic detergent compounds are C<sub>6</sub>-C<sub>22</sub> alkyl phenol-ethylene oxide condensates, generally 5 to 25 EO, i.e. 5 to 25 units of ethylene oxide per molecule, and the condensation products of aliphatic C<sub>8</sub>-C<sub>18</sub> primary or secondary linear or branched alcohols with ethylene oxide, generally 5 to 40 EO.

Suitable anionic detergent compounds which may be used are usually water-soluble alkali metal salts of organic sulphates and sulphonates having alkyl radicals containing from about 8 to about 22 carbon atoms, the term alkyl being used to include the alkyl portion of higher acyl radicals. Examples of suitable synthetic anionic detergent compounds are sodium and potassium alkyl sulphates, especially those obtained by sulphating higher C<sub>8</sub>-C<sub>18</sub> alcohols, produced for example from tallow or coconut oil, sodium and potassium alkyl C<sub>9</sub>-C<sub>20</sub> benzene sulphonates, particularly sodium linear secondary alkyl C<sub>10</sub>-C<sub>15</sub> benzene sulphonates; and sodium alkyl glyceryl ether sulphates, especially those ethers of the higher alcohols derived from tallow or coconut oil and synthetic alcohols derived from petroleum. The preferred anionic detergent compounds are sodium C<sub>11</sub>-C<sub>15</sub> alkyl benzene sulphonates and sodium C<sub>12</sub>-C<sub>18</sub> alkyl sulphates. Also applicable are surfactants such as those described in EP-A-328 177 (Unilever), which show resistance to salting-out,

the alkyl polyglycoside surfactants described in EP-A-070 074, and alkyl monoglycosides.

Preferred surfactant systems are mixtures of anionic with  
5 nonionic detergent active materials, in particular the  
groups and examples of anionic and nonionic surfactants  
pointed out in EP-A-346 995 (Unilever). Especially preferred  
is surfactant system which is a mixture of an alkali metal  
salt of a C<sub>16</sub>-C<sub>18</sub> primary alcohol sulphate together with a  
10 C<sub>12</sub>-C<sub>15</sub> primary alcohol 3-7 EO ethoxylate.

The nonionic detergent is preferably present in amounts  
greater than 10%, e.g. 25-90% by weight of the surfactant  
system. Anionic surfactants can be present for example in  
15 amounts in the range from about 5% to about 40% by weight of  
the surfactant system.

The detergent composition may take any suitable physical  
form, such as a powder, a tablet, an aqueous or non aqueous  
20 liquid, a paste or a gel. The fusion protein according to  
the invention will generally be used as a dilution in water  
of about 0.05 to 2%.

The detergent composition in accordance with the invention  
25 comprising the fusion protein can have any suitable form,  
i.e. the form of a granular composition, a liquid or a  
slurry of the enzyme, or with carrier material (e.g. as in  
EP-A-258 068 and the Savinase (TM) and Lipolase (TM)  
products of Novo Nordisk). A good way of adding the fusion  
30 protein to a liquid detergent product is in the form of a  
slurry containing from 0.005 to 50 % by weight of the  
complex in an ethoxylated alcohol nonionic surfactant.

The detergent compositions of the invention comprise about  
35 0.001 to 50 mg, preferably from 0.01 to 10 mg of fusion  
protein per liter of the rinse liquor in use. A concentrated



detergent composition before use will comprise about 1 to 1000 mg/l, preferably from 10 mg to 100 mg per liter of the detergent product.

5 The invention will now be further illustrated in the following, non-limiting Examples. In the accompanying drawings:

Figure 1 illustrates the ability of two isolated clones to bind to melamine and a variety of other antigens,

10 Figure 2 illustrates the dilution factors used to demonstrate the antibody / antigen binding event of four chosen antibody proteins,

Figures 3 and 4 illustrate the ability of the fusion protein to enhance the delivery of melamine based micro-capsules  
15 (containing fragrance notes) to cotton fabric both in a water rinse formulation (Figure 3) or in a formulated OMO washing powder rinse (Figure 4),

Figure 5 gives the gene sequences of three melamine-binding proteins VhhM-1E7, VhhM-1C8, VhhM-1G711 which were isolated  
20 out of the antibody library.

### Example 1

#### **Identification of melamine capsule binders**

25 Figure 1 illustrates the ability of isolated clones to bind to melamine and a variety of other antigens. With such a small repeating epitope in the melamine particle structure, the very fact that we have isolated binders is surprising. Interestingly the binders also cross-react with gelatin  
30 cross-linked microspheres, suggesting that the binding epitope may also be present in these particles in the form of an amine side chain or a common cross-linked motif whereby formaldehyde or urea cross-linking is common with amine containing micro-particles. Figure 2 illustrates the  
35 dilution factors used to demonstrate the antibody / antigen binding event of the chosen antibody proteins. Figure 5

gives the gene sequences of the 3 melamine binding proteins isolated out of the antibody library.

**Example 2**

5 **Binding perfumed melamine particles to cotton.**

Figures 3 and 4 illustrate the ability of the fusion protein to enhance the delivery of melamine based microcapsules (containing fragrance notes) to cotton fabric both in a water rinse formulation (figure 3) or in a formulated OMO  
10 washing powder rinse (figure 4). OMO is a commercially available laundry detergent powder.



CLAIMS

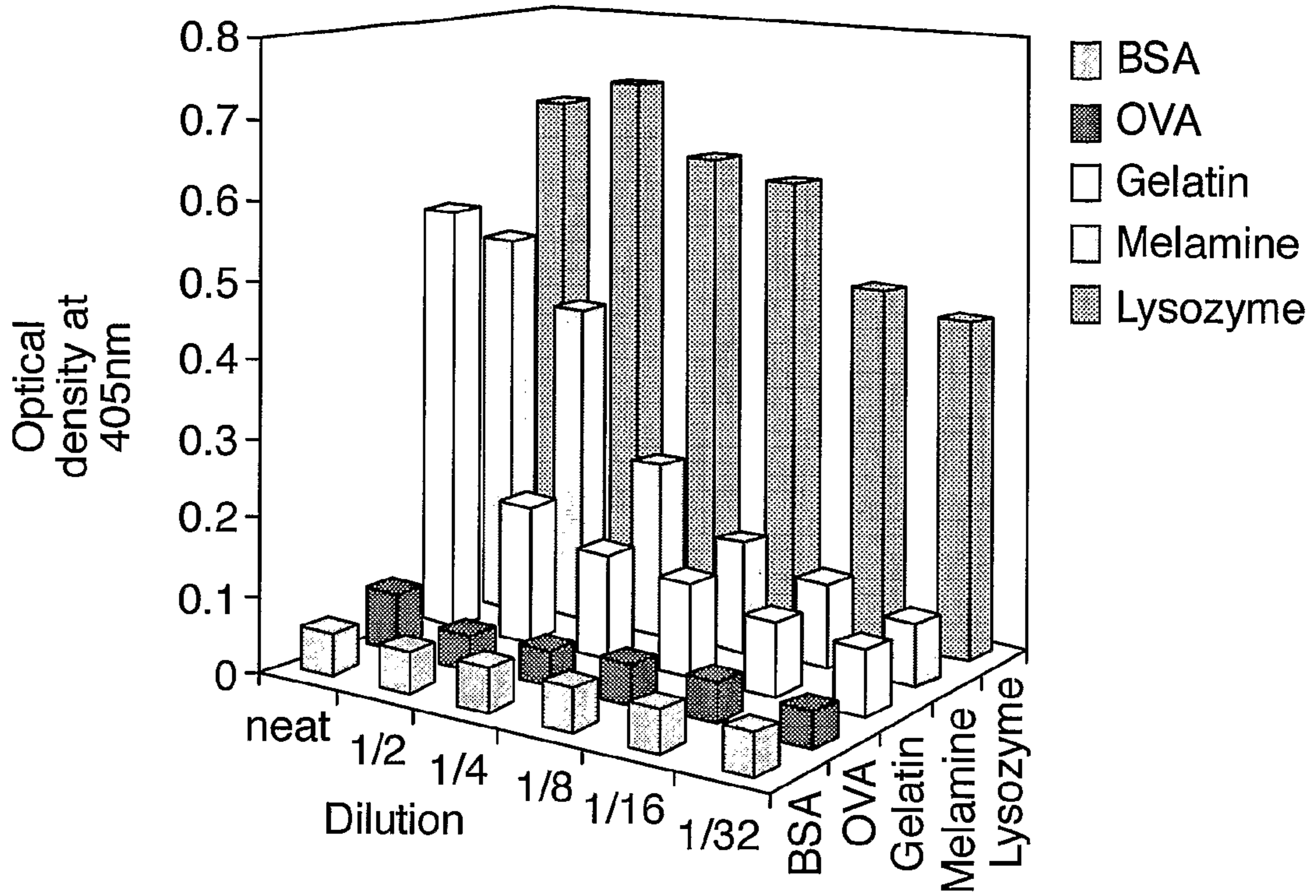
1. Fusion protein comprising a Carbohydrate Binding Domain and a domain having a high binding affinity for a  
5 microcapsule comprised of, or containing, a melamine based chemical component.
2. Fusion protein according to claim 1, wherein the Carbohydrate Binding Domain is a Cellulose Binding Domain.  
10
3. Fusion protein according to any one of the preceding claims, wherein the Carbohydrate Binding Domain is a Cellulose Binding Domain obtainable from a fungal enzyme origin such as Humicola, Trichoderma, Thermomonospora,  
15 Phanerochaete, Aspergillus, Meripilus or from a bacterial enzyme origin such as Bacillus, Clostridium, Streptomyces, Cellulomonas and Pseudomonas.
4. Fusion protein according to any one of the preceding  
20 claims, wherein the Cellulose Binding Domain is obtainable from Trichoderma, Meripilus or Humicola.
5. Fusion protein according to any one of the preceding claims, wherein the domain having a high binding affinity is  
25 an antibody or antibody fragment.
6. Fusion protein according to any one of the preceding claims, wherein the domain having a high binding affinity is a Heavy Chain antibody as found in Camelidae.  
30
7. Fusion protein according to any one of the preceding claims, wherein the domain having a high binding affinity is a peptide.
- 35 8. Fusion protein according to any one of the preceding claims, wherein the Cellulose Binding Domain is connected to

the domain having a high binding affinity for the melamine-type polymer by means of a linker consisting of 2-15, preferably 2-5 amino acids.

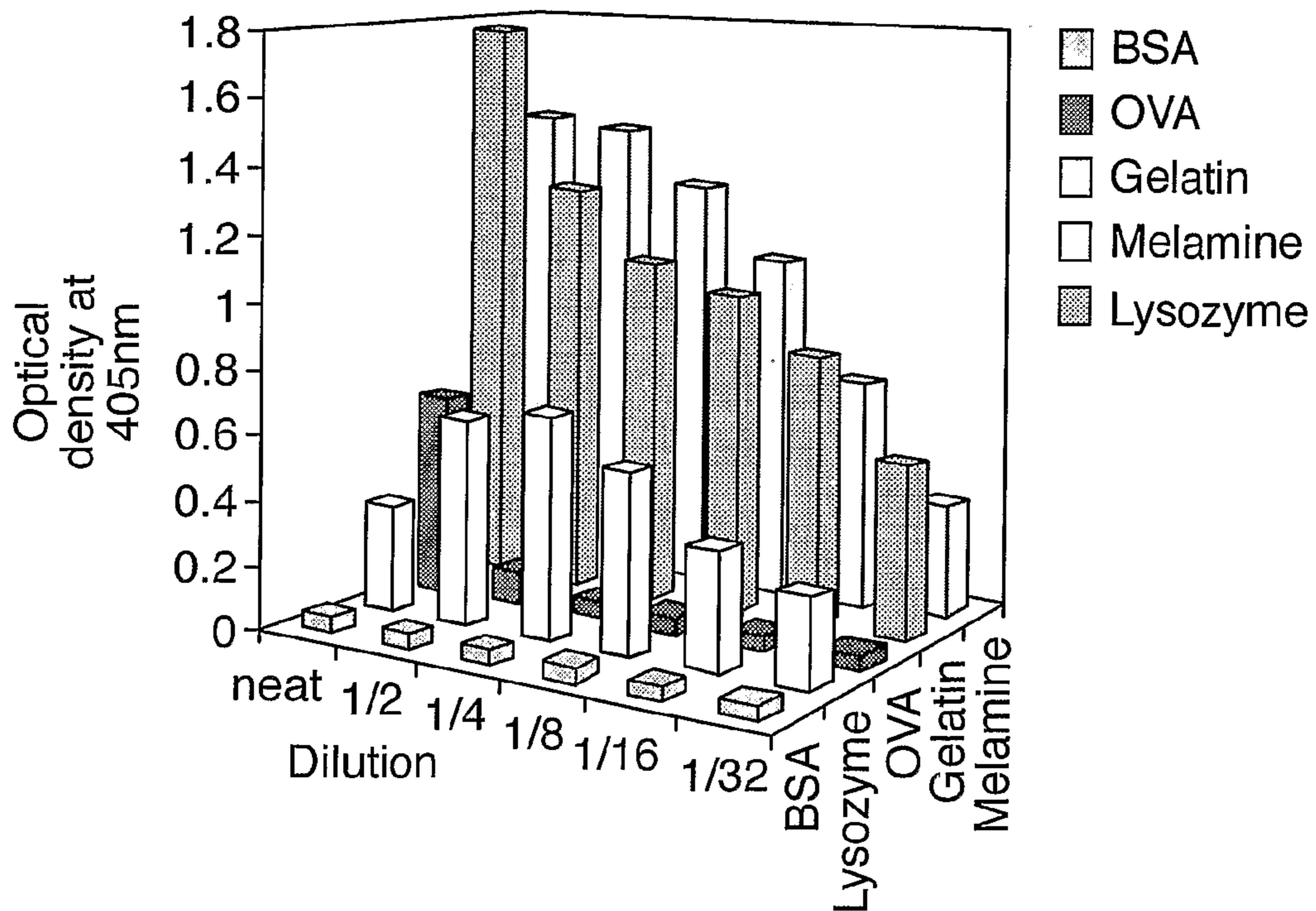
- 5 9. DNA sequence coding for melamine-binding proteins VhhM-1E7, VhhM-1C8 or VhhM-1G711.
- 10 10. Detergent composition comprising one or more surfactants and a fusion protein according to any one of claims 1-8 and micro-particles capsule comprising a melamine-type polymer.
- 15 11. Detergent composition according to claim 10, wherein the micro-particles comprise a benefit agent selected from the group consisting of a fabric softening agents, fragrances, perfumes, polymeric lubricants, photoprotective agents, dye fixative agents, antioxidants, insecticides, soil repelling agents or a soil release agents.
- 20 12. Detergent composition according to claim 11, wherein the benefit agent is a perfume.
- 25 13. Process for delivering a benefit agent to a fabric by treating said fabric with a composition comprising a fusion protein according to any one of claims 1-8 and micro-capsules comprising a benefit agent selected from the group consisting of softening agents, finishing agents/ protective agents, fragrances and bleaching agents.



1/5  
**Fig. 1.** Clone M-1E3 vs. various antigens



**Fig. 2.** Clone M-1E7 vs. various antigens



2/5

Fig.2a.

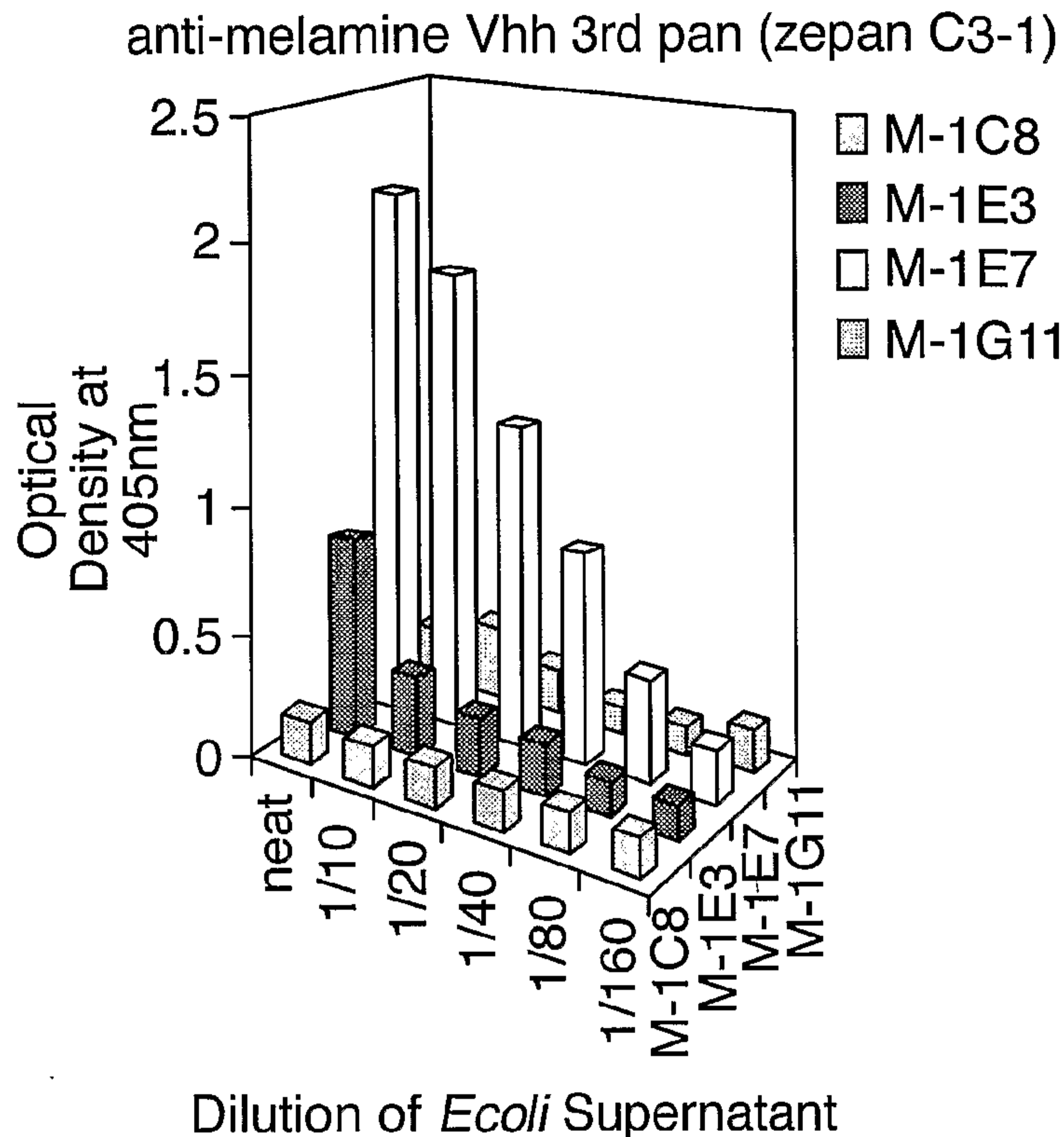
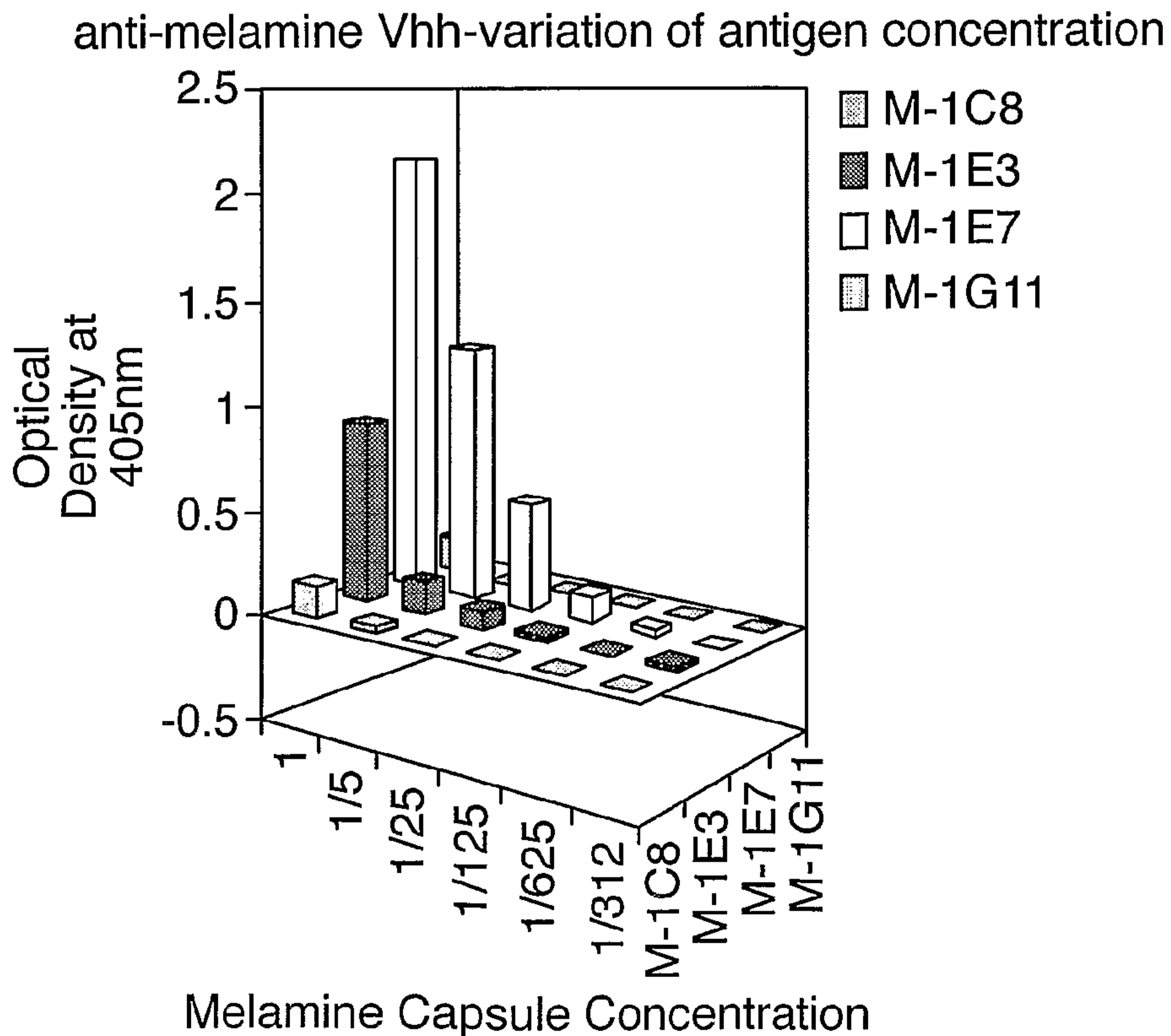


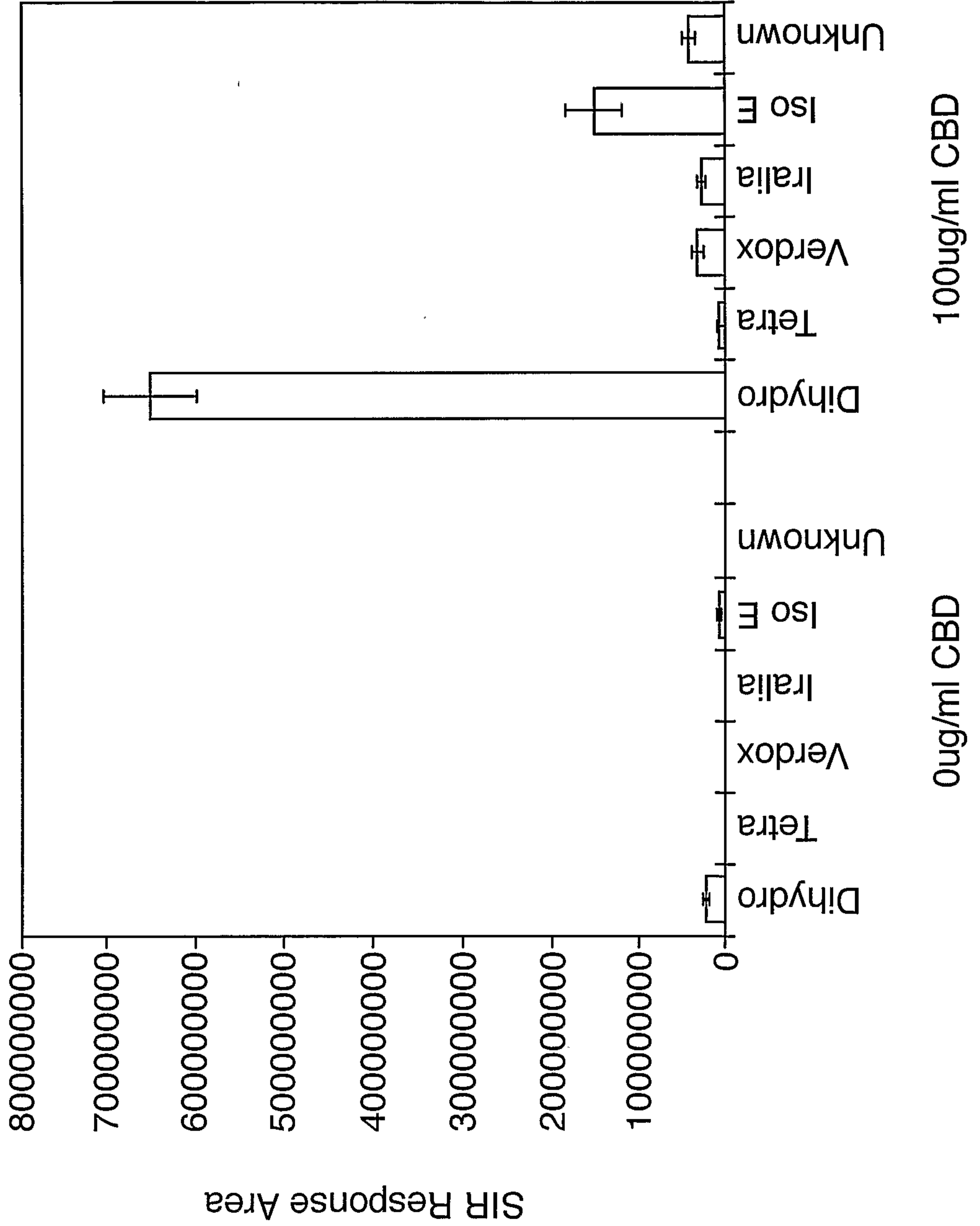
Fig.2b.





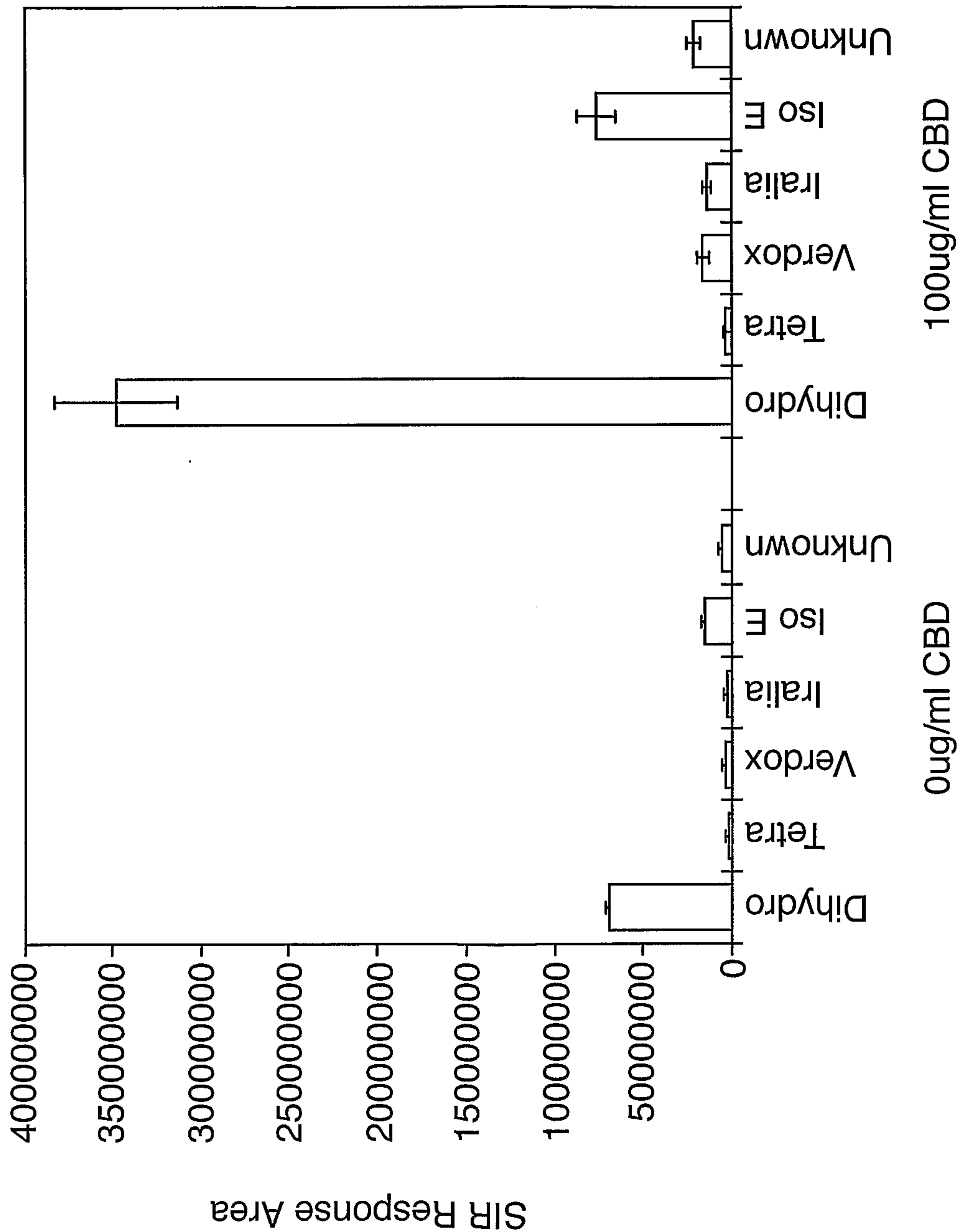
**Fig.3.**

Deposition of Blue Melamine Particles onto NF Cotton using  
Anti-Melamine CBD Fusion, Water Rinse  
(Perfume Detected using GC-MS)



**Fig.4.**

Deposition of Blue Melamine Particles onto NF Cotton using  
Anti-Melamine CBD Fusion, Omo Base Rinse  
(Perfume Detected using GC-MS)





5/5

## Fig.5.

## VhhM-1E7

```

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGCTCTCTGAGACTCTCCTGTGCAGCCTCTGGACG 80
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CACCGGTAGTAGTTATGCATGGGCTGGTTCCGCCAGGCTCCAGGGAAGGAGCGTGAGTTTGTAGCGTCTATTAGCCGGA 160
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
GCGGTAGTAGGACATATTATGCAGACTCCGTGAAGGGCCGATTACCCATCTCCAGAGACAACGC CAAGAACACGGTGTAT 240
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CTGCAAATGAGCAGCCTGAAACCTGAGGACACGGCCGTTTATTACTGTAATTACGGAAGAATAACGCGCGATTATTCGAA 320
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCAGC 359
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

```

## VhhM-1C8

```

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGCTCTCTGAGACTCTCCTGTGCAGCCTCTGGACG 80
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CACCGGTAGTAGTTATGCATGGGCTGGTTCCGCCAGGCTCCAGGGAAGGAGCGTGAGTTTGTAGCGTCTATTAGCCGGA 160
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
GCGGTAGTAGGACATATTATGCAGACTCCGTGAAGGGCCGATTACCCATCTCCAGAGACAACGC CAAGAACACGGTGTAT 240
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CTGCAAATGAGCAGCCTGAAACCTGAGGACACGGCCGTTTATTACTGTAATTACGGAAGAATAACGCGCGATTATTCGAA 320
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCAGC 359
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

```

## VhhM-1G11

```

GCGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGTCTCTGAGACTCTCCTGTGCAGCCTCTGGAA G 80
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CATCACCAGTATGAATGCATGGCCTGGTACCGCCAGGCTCCAGGGAAGCAGCGCGAGTTGGTGCAGCTATTA CTAGGA 160
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
GTGGTAACACAAA CTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAA GAACA CGGTGTATCT G 240
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CAAATGAA CAGCCTGAAA CCTGAGGACA CGGCCGTCTA T TACTGTAAA CAGTGGCTTC CGCGC GCTACTGGGGCCAGG G 320
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
GACCCAGGTCACCATCTCCTCAGC 344
-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

```