Compositions of A-Beta Peptide and Processes for Producing Same

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates generally to pharmaceutical compositions comprising proteins, which are useful in raising a mammalian antibody response. More specifically, this invention relates to pharmaceutically acceptable compositions comprising an amount of amyloid beta peptide effective to elicit an immunogenic response in a mammal and a pharmaceutically acceptable diluent. Preferably, the diluent is a sterile parenterally acceptable aqueous phase.

State of the Art

Amyloid-beta peptide, also known as A-beta or Aβ peptide, is a cleavage product of the amyloid precursor protein (APP). It is the principal component of amyloid plaques in the mammalian brain that are fundamentally involved in and characteristic of Alzheimer's disease. Aβ peptide is a 39-43 amino acid chain varying in length owing to the variability of its processing from APP by several proteases.

Several mutations within the APP protein have been correlated with the presence of Alzheimer's disease. See, e.g., Goate et al, Nature 349, 704)(1991) (valine^{717} to isoleucine); Chartier Harlan et al. Nature 353, 844 (1991) (valine^{717} to glycine); Murrell et al., Science 254, 97 (1991) (valine^{717} to phenylalanine); Mullan et al., Nature Genet. 1, 345 (1992) (a double mutation changing lysisne^{595}-methionine^{596} to asparagine^{595}-leucine^{596}). Such mutations are thought to cause Alzheimer's disease by increased or altered processing of APP to Aβ peptide, particularly processing of APP to increased amounts of Aβ42 and Aβ43. Mutations in other genes, such as the presenilin genes, PS1 and PS2, are thought indirectly to affect processing of APP to generate increased amounts of Aβ42 and Aβ43 (see Hardy, TINS 20, 154 (1997)). The observations indicate that Aβ peptide, and particularly Aβ42, is a causative element in Alzheimer's disease. In the brain, the Aβ peptide aggregates and forms the amyloid deposits comprising the peptide organized into fibrils of β-pleated sheet structures.

The impetus of recent therapeutic research on the treatment or prevention of Alzheimer's disease has focused on halting or slowing the production of Aβ peptide in the brain or in blocking its post-release processing or deposition into amyloid plaques. One therapeutic approach of particular importance to the application of this invention is the use of Aβ peptide in inducing an immune response of the body against it. See, for example, PCT Publication No. WO99/27944, which is incorporated by reference in its entirety for all purposes.

The present invention is directed to novel and unexpected methods for practicing the invention described in PCT Publication No. WO99/27944. In particular, it involves administering certain formulations of the long forms of Aβ peptide to the patient to induce an immune response. However, as has been noted in the art, the longer forms of Aβ peptide are difficult to solubilize in conventional formulation systems.

Specifically, Hilbich, et al., *J. Mol. Biol.*, **218** (1), pp. 149-64, (1991) report that while Aβ1-43 peptide is soluble to a degree in pure water, the addition of ionic components, such as buffers or salts, or organic solvents cause the peptide to precipitate out of solution in the form of an amorphous aggregate. For example, Hilbich found phosphate-buffered saline ("PBS," which in this instance contained 137mM NaCl, 3 mM KCl, 8 mM $Na_2HPO_4 \cdot 2H_2O$ and 2 mM KH_2PO_4 at pH 7.5) rendered 90-94% of the peptide in the composition insoluble. PBS is a conventional carrier for parenteral compositions, approximating the tonicity and pH level of the living system. Five (5) mM NaCl caused 42-50% of the peptide to precipitate. (Ibid., p.153, Table 2). The solution of peptide in pure water would be hypotonic and the pH of such a solution was determined to be 5.5 by Hilbich (idem.). Typically, the pH of the blood supply in man is about 7.4. Dyrks, et al. have also reported Aβ42 is insoluble at physiological conditions. Dyrks, T., Weidemann, A., Multhaup, G., et al. *EMBO J.*7, p. 949-57 (1988).

The conformation of Aβ peptide in the solution can be measured using circular dichroism (C.D.) spectroscopy. Conformational studies of Aβ peptide and fragments using C.D. are reported by Hilbich, et al, idem. See also, monograph by M. Manning entitled: *Protein Structure and Stability Assessment by Circular Dichrosim Spectroscopy,* from Biocatalyst design for Stability and Specificity; Himmel, M. E. and Georgiou, G., eds., ACS Symposium Series 516 (1993) at p. 36. This reference,

hereinafter referred to as the Manning reference, is hereby incorporated by reference in its entirety for all purposes.

Kline, et al., U.S. Patent Nos. 5,851,996 ('996 patent) and 5,753,624 ('624 patent) describe administration of very minute amounts (10^{-2}mg or less) of Aβ peptide or a fragment thereof administered sublingually in a liquid or solid carrier, such as a phenylated saline solution. The '996 patent states that the amyloid beta protein "exists in various structural forms" (col. 2, line 31) that can be used to treat Alzheimer's disease. It is not elsewhere defined what is meant by various structural forms, neither are any characterized beyond the 28 amino acid fragment used in the examples. The doses of Aβ peptide are stated in the '996 patent to be from 10^{-10} to 10^{-2} mg (col. 8, lines 442-43).

In view of the above, the prior art has demonstrated the difficulty in dissolving and maintaining dissolution of Aβ peptide. Additionally, the lack of solubility of the long forms of Aβ peptide has presented difficulties from a sterilization and standardization standpoint. Most standard methods of sterilization are incompatible with peptide formulations, including irradiation, autoclaving and chemical sterilization techniques, such as ethylene oxide gas and glutaraldehyde, all of which cause peptide degradation. Therefore, filtration of the peptide would be the method of choice for sterilization of a formulation of Aβ peptide. Unfortunately, the insolubility of Aβ peptide causes clogging of the filtration membranes and prevents recovery of sufficient quantities of Aβ peptide for a commercial scale process.

SUMMARY OF THE INVENTION

This invention is directed to the surprising and unexpected discovery that aqueous solutions comprising high concentrations of Aβ peptide can be prepared by adjusting the acidity/basicity of such an aqueous solution to a pH effective to solubilize Aβ peptide. Preferably, the pH is adjusted to a pH range of from about 8.5 to about 12, and more prefereably from about pH 9 to 10.

This invention is further directed to the discovery that the solubilized solutions of Aβ peptide lend themselves to sterile filtration through a suitable micropore filter with at least 50% recovery of the Aβ peptide after the sterile filtration. Preferably, at least about 70% of Aβ peptide is recovered after sterile filtration and, more preferably at least 90%. Such sterile solutions can be formulated as pharmaceutical compositions comprising a sufficient amount of Aβ peptide to effect an immunogenic response when administered to a mammal. Preferably, such administration is by parenteral administration, in the form of a suspension composition.

Thus, in one aspect of the composition invention, after sterile filtration the pH of the composition is adjusted to a physiologically acceptable pH to form a peptide suspension containing at least 0.1 mg/ml of Aβ peptide. The composition being useful for parenteral administration. The pH of a suspension composition is between about pH 5 and about 7, preferably between 5.5 and 6.5. A more preferred composition comprises a sufficient amount of QS-21 in conjunction with Aβ peptide to form a visually clear, sterile suspension.

This invention is still further directed to the discovery that the solubilized and sterile solutions of Aβ peptide can be lyophilized to provide for lyophilized formulations comprising Aβ peptide. These compositions can be reconstituted at the appropriate time to provide for an aqueous composition comprising Aβ peptide.

In another of its composition aspects, this invention is directed to an aqueous solution comprising at least 0.01 mg/ml of Aβ peptide wherein said aqueous solution is maintained at a pH sufficient to solubilize said Aβ peptide. Preferably, the solution is maintained at such a suitable pH by use of an effective amount of a pharmaceutically acceptable buffer.

In another of its composition aspects, this invention is directed to a sterile aqueous solution comprising at least 0.01 mg/ml of Aβ peptide wherein said aqueous solution is maintained at a pH sufficient to solubilize said Aβ peptide. Preferably, the solution is maintained at such a pH by use of an effective amount of a pharmaceutically acceptable buffer.

In still another of its composition aspects, this invention is directed to lyophilized compositions comprising a lyophilized composition comprising Aβ peptide which composition is prepared by the process of:

a) freezing a sterile aqueous solution comprising at least 0.01 mg/ml of Aβ peptide wherein said aqueous solution is maintained at a pH sufficient to solubilize said Aβ peptide; and

b) lyophilizing the frozen composition prepared in a) above.

Preferably, the compositions of this invention comprise a long form (as defined below) of Aβ peptide. More preferably the composition comprises a pharmaceutically acceptable buffer which is preferably selected from the group consisting of amino acids, salts and derivatives thereof; pharmaceutically acceptable alkalizers, alkali metal hydroxides and ammonium hydroxides, organic and inorganic acids and salts thereof; and mixtures thereof.

In yet another of its composition aspects, this invention is directed to a composition comprising an aqueous solution comprising at least 0.01 mg/ml of Aβ peptide wherein said aqueous solution is maintained at a pH sufficient to solubilize said Aβ peptide and further wherein said Aβ peptide is substantially in a random coil conformation.

The compositions of this invention can be formulated into pharmaceutical compositions suitable for delivery to a mammal having Alzheimer's disease or at risk of developing Alzheimer's disease. The composition aspects of the invention are directed to pharmaceutical compositions which are in a soluble random coil conformation of Aβ peptide or a stable, aqueous suspension of at least 0.1 mg/ml of Aβ peptide suspended in said composition, or a lyophilized composition, any or all of which may be sterile and pareterally administerable.

In one of its process aspects, this invention is directed to a process for preparing a sterile composition of a long form of Aβ peptide comprising:

adjusting the pH of an aqueous solution sufficient to solubilize the Aβ peptide therein;

dissolving into the solution an amount of the Aβ peptide sufficient to achieve an immunogenic concentration for a mammal;

filtering the resulting solution through a uniform pore size membrane, said pore size being in a range capable of excluding bacteria and passing substantially all of the Aβ peptide through the membrane; and

optionally, for solutions containing 0.1 mg/mL or more of Aβ peptide, adjusting the pH of the resulting solution to between about pH 5 to about pH 7 to obtain a peptide suspension.

In another of its process aspects, this invention is directed to a process for preventing or treating Alzheimer's disease in a mammal which method comprises administering to said mammal a sufficient amount of a sterile aqueous composition comprising at least 0.05 mg/ml of Aβ peptide to induce an immunogenic response in said mammal.

Most preferably, the filtration processes of this invention employ Aβ peptide which is substantially in the random coil conformation.

Brief Description of the Drawings

Figure 1 is a C.D. spectrum showing the mean residue ellipticity measurement plotted as a function of wavelength for two different solutions of Aβ42. The dotted line shows the absorbance at pH 6 and is attributable to the β-pleated sheet form of the molecule. The solid line is a plot of the absorbance of a solution of Aβ42 at pH 9 and is indicative of the random coil conformation of the peptide.

Figure 2 is a plot of Aβ42 placed into a solution against the peak area calculation for the amount of dissolved peptide as determined by reverse phase high performance liquid chromatography, demonstrating the solubility of Aβ42.

Detailed Description of the Invention

This invention is directed to compositions and methods employing aqueous compositions comprising therapeutically effective concentrations of Aβ peptide. However, prior to discussing this invention in further detail, the following terms will first be defined.

Definitions:

The term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 65 percent sequence identity, preferably at least 80 or 90 percent sequence identity, more preferably at least 95 percent sequence identity or more (*e.g.*, 99 percent sequence identity or higher). Preferably, residue positions which are not identical differ by conservative amino acid substitutions.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. A suitable reference sequence would be the human Aβ peptide sequence, specifically the 42 amino acid sequence as reported below. Other suitable forms would be the truncated forms: such as Aβ39, or the extended form, Aβ43 (with an additional threonine group at the C-terminal end). When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally* Ausubel *et al., supra*). One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al., J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST

analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Typically, default program parameters can be used to perform the sequence comparison, although customized parameters can also be used. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89, 10915 (1989)).

For purposes of classifying amino acid substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): norleucine, met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

APP^{695}, APP^{751}, and APP^{770} refer, respectively, to the 695, 751, and 770 amino acid residue long polypeptides encoded by the human APP gene. See Kang et al., *Nature* 325, 773 (1987); Ponte et al., *Nature* 331, 525 (1988); and Kitaguchi et al., *Nature* 331, 530 (1988). Amino acids within the human amyloid precursor protein (APP) are assigned numbers according to the sequence of the APP770 isoform.

In the present invention and in the literature, the weight of Aβ peptide represents about 70% to about 85% Aβ peptide and about 15% to about 30% salt and water. This is determined by amino acid analysis and/or elemental nitrogen analysis. For example, when 0.1 mg Aβ42 peptide is corrected for the peptide content, this represents 0.075 mg of Aβ42 peptide and 0.025 mg water and salt; 0.6 mg Aβ40 peptide represents 0.45 mg of Aβ40 peptide and 0.15 mg of water and salt; and 2.0 mg Aβ42 peptide represents 1.5 mg of Aβ42 peptide and 0.5 mg of water and salt.

Aβ peptide, as used in this invention, refers to those segments of the Aβ peptide capable of forming a β-pleated sheet conformation and raising an immunogenic response when administered either alone or in conjunction with an adjuvant to a mammal. It is within the ordinary skill of the art to determine β-pleated sheet conformation as

described herein by use of, for example, circular dichroism measurements. Immuongenicity may be determined as described in the Biological Activity sections of the Examples given below.

The term "long forms of Aβ" peptide includes any of the naturally occurring forms of Aβ38, Aβ39, Aβ40, Aβ41, Aβ42, and Aβ43 and peptide sequences substantially identical thereto, and preferably the human forms. Aβ39, Aβ40, Aβ41, Aβ42 and Aβ43 refer to Aβ peptide containing amino acid residues 1-39, 1-40, 1-41, 1-42 and 1-43, respectively, amino acids being truncated from the C-terminal end of the peptide. Thus, Aβ41, Aβ40 and Aβ39 differ from Aβ42 by the omission of Ala, Ala-Ile, and Ala-Ile-Val respectively from the C-terminus, as can be seen by reference to the Aβ peptide sequence below. Aβ43 differs from Aβ42 by the presence of a threonine residue at the C-terminus. The sequences of these peptides and their relationship to APP are illustrated in Figure 1 of Hardy et al., TINS 20, 155 (1997).

Aβ42 has the sequence: H2N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-OH.

The term "long forms of Aβ" peptide also includes analogs thereof. Analogs include allelic, species and induced variants. Analogs typically differ from naturally occurring peptides at one or a few positions, often by virtue of conservative substitutions. Analogs typically exhibit at least 80 or 90% sequence identity with natural peptides. Some analogs also include unnatural amino acids or modifications of N or C terminal amino acids. Examples of unnatural amino acids are α, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methyl-histidine, 5-hydroxylysine, ω-N-methylarginine.

Aβ may be utilized in the compositions and processes of the invention from about 0.05 mg/ml to its upper solubility limit of about 2.0 mg/ml (referring to Fig.2). Preferred ranges of the peptide are from about 0.1 to about 0.8 mg/ml and more preferred is a range of from about 0.3 to about 0.6 mg/ml.

It has been reported that the insoluble form of Aβ peptide found in amyloid plaques is in the β-pleated sheet conformation. Soto, C., et al., *Neuroscience Letters*, **186** (2-3), pp. 115-118, (1995). Also, Simmons, L.K., et al. Molec. Pharmacol., 45 (3) pp. 373-379 (1994) have reported that the β-sheet conformation is associated with the neurotoxic effects of the peptide, while the random coil form is only weakly toxic or inactive. As noted above, the methods and compositions of this invention may employ a random coil conformation of Aβ peptide. Applicants demonstrate herein that the random coil conformation is capable of raising an immunogenic response in test mammals. The random coil conformation is most preferred in the microfiltration of the processes.

The term "random coil" refers to an open chain conformation of the Aβ peptide. The random coil is a secondary conformation of the peptide backbone and is not ordered into a regular conformation such as the α-helix or β-pleated sheet forms. Rather the random coil is disordered from the hydrophobic folding and hydrogen bonding interactions that characterize the other, more regularly arranged forms. A random coil may still possess some turns of the peptide backbone or partial ordering, however, such features are random and dynamic, and thus are not typical of all the random coil population. In the random coil conformation, the peptide exhibits good solubility and filterability. The α-helix and β-pleated sheet conformations are well-known peptide conformations in the art. They are described, for example, in Lehninger's Biochemistry (2nd Ed.,Worth Publishers, 1975) at pp. 128-9 for the α-helix and for the β-pleated sheet at pp. 133-4, which is incorporated by reference herein.

The random coil conformation of Aβ peptide can be characterized by its circular dichroism ("CD") spectrum and is easily distinguished from the β-pleated conformation. As represented by a CD spectrum, a random coil is typified by a strong negative band between 190 and 200 nm, and little CD signal is seen at wavelengths longer than 215 nm. If there is a CD signal at longer wavelengths is very weakly positive, centered at 220-225 nm. This is shown in Figure 1 of the Drawings. The β-pleated sheet conformation on the other hand displays a sharp and strong positive band centered at about 200 nm. For a review of peptide secondary structure elucidation by C.D. spectra, please refer to the Manning reference cited supra.

Aβ peptide is substantially in a random coil conformation when greater than 50% of the Aβ peptide is the random coil conformation. Preferably, greater than 70% or 80%, and most preferably, greater than 85% or 90% of the Aβ peptide is in the random coil conformation.

"Parenteral compositions" are those compositions which are sterilized and suitable for administration directly into the body by, for example, injection or infusion, i.e. by routes which have immediate contact with the blood and without the barrier or immune system protections afforded by administration forms that enter the body via the dermal, mucosal, digestive or respiratory systems. For these reasons, sterility is a necessity for a parenteral composition.

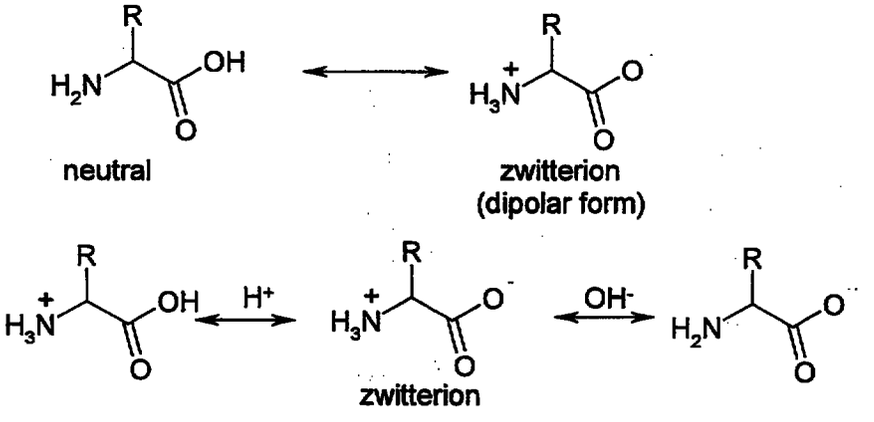
"Infusion", as a means of drug administration, involves a substantially continuous and slow flow of a drug solution into the blood stream over a relatively long period. "Injection", on the other hand, is a rapid administration of a unit dose of a solution or suspension.

Intravascular (or intravenous or IV); intramuscular (IM); intraperitoneal (IP); subcutaneous (SC) ; and intrasternal all refer to different modes of adminstration of parenteral compositions. They describe in anatomical terms the area of the body in which the parenteral composition is introduced by injection or infusion. It is contemplated that the compositions of the present invention having a physiologically acceptable pH can be administered by any of the above modes, depending on the individual patient and the judgment of the administering physician. The higher pH solutions (pH > 8) may most advantageously be administered by a slow IV infusion or IV drip.

In understanding the terms "buffer" and "buffering agent" as used in the invention, it should be recalled that the titration curve of an acid or base has a relatively flat zone extending to about 1.0 pH unit on either side of the titration midpoint. At the midpoint, an equivalent amount of the proton-donor and proton-acceptor species of the acid or base are present. In this zone, the pH of the system changes relatively little when small increments of H^+ or OH^- are added. This is the zone in which a conjugate acid-base pair acts as a buffer, a system which tends to resist change in pH when a given increment of H^+ or OH^- is added. At pH levels outside this zone there is less capacity of

the buffer to resist changes to the pH. A buffer's power is maximum at the pH of the exact midpoint of its titration curve, i.e., where there are equal concentrations of the proton acceptor and proton donor and the pH is equal to the pK' (the acid dissociation constant). Buffer preparations are described in detail in Data for Biochemical Research, Rex, M.C., Oxford Science Publications, 1995.

Many physiological mechanisms operate within the body to maintain blood pH within the narrow limits of 7.35 to 7.45. While some of the major buffering mechanisms are based on carbonic acid or phosphoric acid equilibria, many other mechanisms involve amino acids and proteins. For example, the pH of tears is maintained at 7.4 by protein buffers. Single amino acids are also useful as buffers, although they show a more complex titration curve on account of having proton donor and proton acceptor atoms within the same molecule. A molecule of this type is referred to as zwitterionic, i.e. it exists in a form having both positively and negatively charged sites within the same molecule. Amino acids are capable of buffering both the addition of H^+ ions or OH^- ions, as shown below.



For the purposes of this invention a "pharmaceutically acceptable buffer" is defined broadly to include conjugate acid – base pairs as well as acidic or basic compounds that have the ability to adjust or to maintain the pH of a solution of Aβ peptide at a desired level. In the solubilization/filtration processes of this invention, that is pH 8.5 or above or in another aspect below pH 4.

Preferably, such buffers are selected from the group consisting of amino acids, salts and derivatives thereof; pharmaceutically acceptable alkalizers, alkali metal hydroxides and ammonium hydroxides, organic and inorganic acids and salts thereof;

and mixtures thereof. Buffering agents are employed in concentrations sufficient to reach and maintain the desired pH and thus the concentrations are dependent on the acidity/basicity of the individual buffer or combination chosen. Selection of an effective concentration is within the ordinary skill of the art, using for example, pH meters and titration techniques.

Examples of classes of compounds useful in the present invention are hydroxides, including alkali metal hydroxides and ammonium hydroxides, alkalizers known in the pharmaceutical arts, including but not limited to tris, sodium borate ($Na_2B_4O_7$) and disodium citrate, amino acids, salts or esters or amides of amino acids and simple derivatives thereof, for example, N-acetyl derivatives of amino acids. Particularly preferred buffers are glycine (e.g., sodium glycinate), arginine and lysine, sodium hydroxide and ammonium hydroxide.

Examples of pharmaceutically acceptable acids for practice of the methods of the present invention are, without limitation, hydrochloric acid, phosphoric acid, citric acid, acetic acid, maleic acid, malic acid and succinic acid, and the like.. Additionally these acids may be used to titrate the pH value of a basic solution to a lower, more physiologically acceptable level, resulting in a peptide suspension composition.

Conversely, basic compounds such as those listed above can be used to titrate a low pH value of a filtered solution to a more physiologically acceptable pH, also resulting in a peptide suspension.

Combinations of the pH adjusting agents are also contemplated. Conjugate acid base pairs as discussed above and exemplified by salts such as ammonium acetate are also within the scope of buffering agents for the invention. Such a conjugate pair could be formed, for example, by the titration of a basic solution of ammonium hydroxide with acetic acid to a more nearly neutral solution of ammonium acetate.

As used herein, the term "tonicity modifier" includes agents that contribute to the osmolality of the solution. Examples of tonicity modifiers suitable in the present invention include, but are not limited to saccharides (sugars) such as mannitol, sucrose and glucose and salts such as sodium chloride, potassium chloride and the like. Preferably, the tonicity agent will be employed in an amount to provide a final osmotic

value of less than about 350 mOsm/kg and more preferably between about 250 to about 350 mOsm/kg, and most preferably between about 280 to about 320 mOsm/kg. It will be noted that the charged compounds that serve as buffers in the formulations can also affect tonicity. Thus the tonicity of a buffered solution of Aβ peptide is first determined before being adjusted further by addition of tonicity modifying agents.

A chelating agent may optionally be used in the processes and compositions of the invention. Examples of preferred chelating agents include ethylenediaminetetraacetic acid (EDTA), and its salts (such as sodium) which are normally employed at a concentration of 0.05 to 50 mM, more preferred at concentrations of 0.05 to 10 mM, or about 0.1 to 5 mM being most preferred. Other known chelating (or sequestering agents) such as certain polyvinyl alcohols can also be used.

Optionally, the compositions may contain a surfactant or detergent such as polysorbate (e.g. Tween®) or 4-(1,1,4,4-tetramethylbutyl) phenyoxypolyethoxyehthanols (Triton®), or polymers of polyethylenepolypropylene glycols (Pluronics®). The surfactant ranges from about 0.005 to 1%, with about 0.02 to 0.75% preferred. A preferred polysorbate is PS-80, which is commercially available as Tween® 80.

Wetting agents are also contemplated as excipeints useful in the invention. The polyethylene glycols, e.g. PEG 3350, are useful in modifying the association of Aβ particles and the solubility thereof by associating with the polymer suface and ordering its hydrophilic moieties in the aqueous phase. Wetting agents may be present form 0.5 to 5 % (w/v).

Pharmaceutically acceptable sugars (for example sucrose, dextrose, maltose or lactose) or pharmaceutically acceptable sugar alcohols (for example mannitol, xylitol or sorbitol) have no influence on the medical effects of an active ingredient. In one aspect of this invention, sugars or sugar alcohols having a molecular weight of less than 500, and capable of easily dispersing and dissolving in water, can be used. Examples of sugars and sugar alcohols usable in the present invention include xylitol, mannitol, solbitol, arabinose, ribose, xylose, glucose, mannose, galactose, sucrose, lactose, and the like. They can be used alone, or as a mixture of two or more of these compounds. The

most preferred sugar is mannitol, especially in the lyophilized compositions; sucrose is also preferred in the solution compositions.

It has also been found that the use of QS-21, an adjuvant, in the compositions of the invention interacts with the suspended protein in such a way that a visually clear suspension can be formed. This is a desirable interaction in that the peptide is in the β-pleated sheet conformation but is suspended in the phase in very small particles and may provide more advantageous properties to the composition, such as added stability of the suspension or improved immunogenic properties. DPPC (dipalmitoyl phosphatidyl choline) is known in the art and is anticipated to interact with Aβ peptide in a similar fashion to QS-21 to provide the same small particle suspension and allow for use of other of the adjuvants in the invention, while providing a visually clear suspension. Alternatively, other adjuvants may be used in admixture with QS-21.

Lyophilization is a technique well known in the pharmaceutical arts, as are techniques for stablizing peptides during and after the lyophilization process. Stabilization of a protein or peptide lyophilisate in an amino acid, saccharide matrix is also known to those of skill in the art. See, for example: Lueckel B., et al., *Formulations of sugars with amino acids or mannitol - Influence of concentration ratio on the properties of the freeze-concentrate and the lyophilizate,* PHARM. DEV. TECHNOL. 3(3) pp. 325-336 (1998). The Royal Pharmaceutical Society of GB Symp: 'New Analytical Approaches to the Characterization of Biotechnology Products', June 1996 Luckel B.; et al., presented: *A strategy for optimizing the lyophilization of biotechnological products,* PHARM. SCI. (UK) 3(1) pp. 3-8 (1997). Both of these references are hereby incorporated by reference in their entirety for all purposes.

The term "pharmaceutically acceptable" modifies any composition to mean that the composition does not impart any deleterious or untoward effect on the subject to which it is administered and in the context in which it is administered. The terms "pharmaceutically acceptable diluent" and "pharmaceutical acceptable excipient" refer to any compound which preserves or does not alter the activity of the active compound(s) and does not impart any deleterious or untoward effect on the subject to which it is administered and in the context in which it is administered, such as a non-toxic pH

adjusting or buffering agent or a tonicity modifying agent or chelating agent, and the like.

Compositions or processes "comprising" one or more recited elements may include other elements not specifically recited. For example, a composition that comprises Aβ peptide encompasses both Aβ peptide in the composition as recited and Aβ peptide as a component of a composition having other non-recited components.

Further definitions are as follows:

Abbreviation	Definition
°C	degrees Centigrade
cc	cubic centimeter
C.D.	circular dichroism
pH	log[H], a measure of the hydryogen ion content and thus the acidity or basicity of a solution
pK'	acid dissociation constant related to pH by: $pH = pk' + \log \frac{[H+ \text{ acceptor}]}{[H+ \text{ donor}]}$
PS80	polysorbate 80 or Tween 80® copolymer of polysorbate and ethylene oxide; Merck Index monograph no. 7559 (11th Ed.)
μm	micron
Min	minute
ml	milliliter
N	normality –indication of molarity of a solution
M	molarity, value stated in moles/liter
mM	millimoles
DMSO	dimethylsulfoxide
EDTA	ethylenediamine tetraacetate, usually as disodium salt
Tris	tromethamine, tris(hydroxymethyl)aminomethane Merck Index monograph no. 9684 (11th Ed.)
RP HPLC	reverse phase high performance liquid chromatography
RPM	revolutions per minute
CFA / IFA	complete Freund's adjuvant / incomplete Freund's adjuvant (Chang et al., Advanced Drug Delivery Reviews 32, 173-186 (1998)
MPL	3-O-deacylated monophosphoryl lipid A (MPL™) (see for example GB 2220211).
QS 21	triterpene glycoside or saponin isolated from the bark of the Quillaja Saponaria Molina tree of South America (see Kensil et al., in Vaccine Design: The Subunit and Adjuvant Approach (eds. Powell & Newman, Plenum Press, NY, 1995); US Pat. No. 5,057,540). (Stimulon™ QS-21)
FIO	for information only

Filtration is the process of removing contaminants by size exclusion from fluids passing through a membrane filter having a uniform pore size. Although micron-sized particles can be removed by use of non-membrane or depth materials such as those found in fibrous media, only a membrane filter, having a precisely defined pore size, can ensure quantitative exclusion of particles having a smaller size. Thus with membrane filtration it is possible to quantitatively remove bacteria from a solution when passed through a microfilter and thus effect sterilization. Previously with Aβ peptide purification the peptide was so insoluble or remained aggregated to the point that the solution could not be microfiltered at a commercial scale owing to blockage of the membrane by particulates and/ or to poor recovery of the peptide in the resulting microfiltered solution.

Preferred filters are generally defined as those filters having the capability to remove particles from 0.2 microns up.

The membrane filter is uniformly cast on a substrate, and generally performs separations based on size exclusion at the membrane surface. However, a surface type membrane filter may trap particles both in the depth of its structure and at the surface of the membrane. In size exclusion separations, particles smaller than the pores of the membrane filter pass through while larger particles are retained at the membrane surface. Because these defined pore size filters do not "unload," (i.e. as the filter begins to fill up with trapped particles, increasing the flow pressure can allow passage of small amounts of trapped solids) they are the devices of choice for microbiological control. "Sterilizing grade" surface type membrane filters are well known in the pharmaceutical industry.

In all filtration applications, the permeability of a filter medium can be affected by the chemical, molecular or electrostatic properties of the filtrate, however it has been found that the hydrophilic microfilters used in the present invention are stable to the high or, conversely, low pH environment depending on the method employed and reliably remove undesired particulate matter without clogging. It is within the skill level of the ordinary skilled artisan to be able to select and use hydrophilic microfilters. Commercially available product specifications or websites such as: http://millispider.millipore.com/corporate/sitemap.nsf/catalogs (as of May 1999) enable the selection of filters having the desired characteristics.

Examples of preferred hydrophilic filters operative in the present invention are Millipore Durapore®, also called Millex GV, (Millipore Corporation, headquartered in Bedford, MA), a polyvinylidene fluoride hydrophilic polymer having good stability and low protein binding characteristics and a 0.22 μm pore diameter; Millex GN™, a hydrophilic nylon material having a 0.2 μm pore size; and Millex GP™, a hydrophilic surface modified polyethersulfone polymer of pore size 0.22 μm. More preferred is the Durapore filter owing to its stability at pH 9-9.5.

Preferably, substantially all of the Aβ peptide is recovered after filtration. Recovering substantially all the Aβ peptide is defined to mean greater than about 50% of the peptide is recovered after sterile filtration. Preferably, greater than 80% of the Aβ peptide is recovered after sterile filtration, and most preferably, greater than 90% is recovered after filtration.

Methods

The methods of this invention involve the preparation of aqueous compositions comprising concentrations of at least 0.01mg/ml of the Aβ peptide. Such compositions are prepared by adjusting the pH of the aqueous solution such that the Aβ peptide will dissolve therein in requisite concentrations (e.g., at a concentration of from about 0.1 mg/ml to about 2.0 mg/ml).

Adjusting the pH of the aqueous solution is accomplished via conventional methods typically exemplified by the addition of either an acid or a base to arrive at the desired pH. Preferably, the acid or base employed is pharmaceutically acceptable at the amounts employed. In order to maintain the pH of the solution over prolonged storage, it is preferable to employ a pharmaceutically acceptable buffer in the composition. The selection of a suitable buffer relative to the desired pH is within the skill of the art.

Preferably, the pH of the aqueous composition is adjusted to between about pH 2 to 4 or about pH 8.5 to 12. At such pHs, the Aβ peptide is surprisingly quite soluble.

When the process of the invention is practiced at low pH (about pH 2 to 4) then acids such as hydrohalide acids (e.g., HCl, HBr), phosphoric acid, citric acid and acetic acid and other pharmaceutically acceptable acids may be employed to lower the pH of the solution to the desired pH. Selection of suitable acids in within the skill of the art.

When the process of the invention is practiced at high pH (about pH 8.5 to 12), then pharmaceutically acceptable bases such as alkali metals, ammomium hydroxides (e.g., NaOH, NH4OH) and the like may be employed to raise the pH of the solution to the desired pH. At high pH, the pH of the solution in a given buffer is preferably adjusted to between 8.5 to 11. More preferred is a pH level of between pH 9 and 10.

Either prior to or subsequent to pH adjustment, requisite concentrations of the Aβ peptide are added to the solution. It is preferred, of course, to add the Aβ peptide after pH adjustment in order to immediately solubilize the peptide. Upon addition, gentle stirring and heating may be necessary to assist in the solubilization process.

The addition of any optional additives such as pharmaceutically acceptable buffers, tonicity agents, adjuvants, etc. to the composition can occur at any convenient time prior to or subsequent the addition of the Aβ peptide.

Once the aqueous solutions described above are prepared, sterile filtration and/or lyophilization can proceed according to procedures well known in the art which procedures are exemplified by the examples below.

The following solutions are preferred buffer systems for solubilizing Aβ peptide at target concentrations ranging from 0.6 to 2.0 mg/ml peptide:

Preferred Amino Acid Compositions:

10 mM Sodium Glycinate , pH 9.0, 9.5, or 10.0
and/or with 0.02 to 1.0 % (w/v) polysorbate 80 (PS-80)
optionally containing one or more tonicity modifiers sufficient for parenteral administration;

10 mM L-Arginine-HCl, or 10 mM L-Lysinate, both at pH 9.0, 10.0

and/or with 0.02 to 1.0 % (w/v) polysorbate 80 (PS-80)
optionally containing one or more tonicity modifiers sufficient for parenteral administration

The compositions of the present invention are preferably stored at low temperature to discourage degradation or aggregation of the peptide chains. The preferred temperature range for storage of the Aβ peptide compositions is 2 to 8° C.

The following examples illustrate practice of the invention. These examples are for illustrative purposes only and are not intended in any way to limit the scope of the invention claimed.

Examples

Example 1 - Peptide Solubility

Peptide and Reagents

Aβ42 as its trifluoroacetate salt was obtained from American Peptide Co., Lot Numbers M05503T1, M10028T1 and used without further modification. Other salts are available and have been successfully employed in the processes of the invention. For examples of alternative counter-ion salts of the Aβ peptide see Table 2.

All acid, base and buffer solutions were prepared from laboratory grade reagents, and stored as sterile filtered stock solutions for convenience. Polysorbate 80 (Tween 80, PS80): 4% stock solutions were prepared by w/v dilution of a qualified low peroxide PS 80 solution (10% solutions of low peroxide polysorbitan mono-oleate are available from Aldrich-Sigma Chemicals, for example).

Peptide Solubilization:

Approx. 500 –700 ug Aβ peptide was weighed and dissolved with the appropriate amount of buffer solution to give a theoretical 0.6, 0.8 or 1.0 mg/ml Aβ42 final concentration. The peptide was directly weighed into 4 ml Wheaton glass vials with screw top caps. The appropriate amount of the buffer solution was added and the peptide was gently mixed for 15 to 30 minutes. Solutions were visually scored for solubility as follows: (+) = poor solubility, cloudy suspension; (++) = clear suspension with some insoluble particulates; (+++) = clear solution.

Table 1. Visual Solubility of Solutions

Buffer Description	Target conc. Aβ42 (mg/ml)	Visual Solubility
0.01 N NaOH	1.0, 0.8	+++
0.01 N NH4OH	1.0, 0.8	+++
50 mM Sodium Glycinate, pH 9.0	1.0	+++
10 mM Sodium Glycinate, pH 9.0	1.0, 0.8, 0.6	+++
10 mM Sodium Glycinate, pH 9.5	0.6	+++
10 mM Sodium Glycinate, pH 10.0	1.0, 0.8	+++
10 mM Sodium Glycinate, pH 9.0, 0.02% PS80	1.0	+++
10 mM Sodium Glycinate, pH 9.5, 0.1 % PS 80, 5 % Sucrose	0.6	+++
10 mM Sodium Glycinate, pH 9.5, 4 % Mannitol, 1 % Sucrose	0.6	+++
10 mM Sodium Glycinate, pH 9.5, 4 % Mannitol	0.6	+++
10 mM Sodium Glycinate, pH 10.0, 0.02% PS80	1.0	+++
10 mM L-Arginine-HCl, pH 9.0	0.6	+++
10 mM L-Arginine-HCl, pH 10.0	0.8	+++
10 mM Sodium L-Lysinate, pH 9.0	0.8, 0.6	+++
10 mM Sodium L-Lysinate, pH 10.0	0.8	+++
10 mM Sodium Lysinate, pH 9.5, 4 % Mannitol	0.6	+++
10 mM Ammonium Acetate, pH 9.0	1.0	+++
10 mM Ammonium Acetate, pH 9.0, 0.02 % PS80	1.0	+++
50 mM Tris-HCl, pH 10.0, EDTA (0.5 mM), 0.02 % PS-80	1.0	+ +
10 mM Sodium Borate, pH 9.0	1.0	+++
10 mM Sodium N-Acetyl-D-Glutamine, pH 9.0	0.8	+++
10 mM Glycine-HCl, pH 3.0	1.0	++
0.01N HCl	1.0	+++
0.01 M Phosphoric Acid	1.0	+++
DMSO (neat)	0.6	+++
10 mM Sodium Glycinate, pH 8.0	1.0	+ +
10 mM L-Arginine-HCl, pH 9.0	0.8, 1.0	++
10 mM Sodium Ammonium Bicarbonate, pH 9.0	0.8	++
10 mM Sodium Ammonium Carbonate, pH 9.0	0.8	++
50 mM Tris-HCl, pH 10.0, EDTA (0.5 mM)	1.0	+ +
10 mM Sodium Borate, pH 10.0	1.0	++
10 mM L-Arginine-HCl, pH 8.0	1.0	+
10 mM Ammonium Acetate, pH 8.0	1.0	+
10 mM Ammonium Acetate, pH 8.0, 0.02 % PS-80	1.0	+
50 mM Tris-HCl, pH 8.0, pH 9.0, or pH 10.0	1.0	+
50 mM Tris-HCl, pH 8.0 or pH 9.0, with 0.5mM EDTA	1.0	+

Buffer Description	Target conc. Aβ42 (mg/ml)	Visual Solubility
50 mM Tris-HCl pH 8.0 or pH 9.0, with 0.5 mM EDTA and 0.02 % PS-80	1.0	+
50 mM or 100 mM NaCl, pH 9.0	1.0	+
50 mM Sodium Phosphate, pH 8.0, pH 9.0 or pH 10.0	1.0	+
50 mM Sodium Glycinate, pH 8.0 or pH 10.0	1.0	+
10 mM Sodium N-Acetyl-D-Glucosamine, pH 9.0 or pH 10.0	0.8	+
10 mM Sodium N-Acetyl-D-Glutamine, pH 10.0	0.8	+
10 mM Sodium Citrate, pH 3.0 or pH 4.0	1.0	+
10 mM Sodium Acetate, pH 4.0	1.0	+

The solutions ranked as +++ were visually clear at the target concentration. The pH range was from pH 9 to pH 10 for the buffered solutions. The pH of the inorganic solutions such as NaOH and NH_4OH were alkaline while HCl and phosphoric acid were strongly acidic. Solubility of the peptide was achieved from 0.6 to 1 mg/ml peptide at pH values approximately > pH 9. Additives such as polysorbate 80, sucrose, mannitol and EDTA did not affect the solubility of the peptide and assist in increasing tonicity and recovery after filtration, or may act as chelating agents. Acidic solutions (approximately pH 4 or below) also solubilize the Aβ42 peptide at 0.6 to 1 mg/ml. Solutions ranked as ++ visually appeared partially soluble when the peptide was dissolved at the target concentration. The peptide may be soluble at a lower concentration than tested herein. Those solutions ranked as + visually did not achieve complete solubility at the target concentration.

Example 2 - Solubility of Aβ peptide in buffered solutions

Aβ42 (0.6, 1.0, 1.5, 2.0, 3.0 and 3.5 mg/ml) was solubilized in 10 mM sodium glycinate buffer, pH 9.0. Each solution was centrifuged in a bench top centrifuge (>10,000 RPM, ~ 10 mins) as cloudy suspensions were observed for concentrations 2.0 - 3.5 mg/ml (solutions of 0.6 - 1.5 mg/ml were visually clear).

An aliquot of the supernatant from each solution was then analyzed by RP HPLC.

Table 2 contains the tabulated peptide peak areas and a graph demonstrating the solubility of Aβ42 in sodium glycinate buffer at pH 9.

Table 2. Solubility Limits, Aβ42 peptide, TFA salt

Aβ42 mg/mL (10mM glycine, pH 9)	HPLC Peak Area
0.6	3613553
1	5921792
1.5	8850393
2	9213446

Addionally, the Aβ42 peptide was purified as the trifluoroacetate, ammonium, chloride and sodium salts. These salts were dissolved in several buffers, at a 0.45 mg/mL Aβ42 peptide concentration, correcting for the counterion contribution of the various salts. Solutions of the peptide were filtered and the recoveries of the peptides were determined by comparing the RP-HPLC peak areas of the peptide before and after filtration. All salts were soluble and readily filtered at 0.45 mg/mL, as shown in Table 3.

Table 3. Solubility of Alternate Salts of the Aβ42 peptide

0.45 mg/mL Aβ42	Recovery (%)
Ammonium salt in 1 mM NH4OH	70
Ammonium salt in 2 mM NH4OH	106
Ammonium salt in 10 mM Sodium Glycinate, pH 9.0	115
Ammonium salt in 10 mM Sodium Glycinate, 5% Sucrose, pH 9.0	96
Ammonium salt in 10 mM Sodium Glycinate, pH 9.5	101
Ammonium salt in 10 mM Sodium Glycinate, pH 10.0	106
Triflouroacetate salt in 10 mM Sodium Glycinate, pH 9.0	101
Chloride salt in10 mM Sodium Glycinate, pH 9.0	101
Sodium salt in 10 mM Sodium Glycinate, pH 9.0	100

Example 3 - Recovery of Solubilized Peptide from Representative Hydrophilic Filters

Syringe filters tested (25mm filter diameters, 3.9 cm^2 filter area): Millex GV, 0.22 uM: a hydrophilic polyvinylidene difluoride (PVDF, Durapore®) membrane with low protein binding properties, Millex GN, 0.20 uM: a hydrophilic nylon membrane with low protein binding properties, and Millex GP, 0.22 uM: a hydrophilic surface-modified polyethenesulfone (PES) membrane with low protein binding properties.

The above listed filters were employed as representatives of the types of commercially available hydrophilic microfilters. The filtration studies were performed using the following solubiliztion systems:

1. 0.6 mg/ml Aβ42 in 0.01 N NH4OH

2. 0.6 mg/ml Aβ42 in 10 mM sodium glycinate, pH 9.0
3. 0.6 mg/ml Aβ42 in 10 mM sodium lysinate, pH 9.0
4. 0.6 mg/ml Aβ42 in 10 mM Arginine –HCl, pH 9.0

Approximately a 2 ml volume of each Aβ42 solution was filtered over each filter. Peptide concentrations were quantitated by reverse phase chromatography (RP HPLC). Filter recoveries were determined by comparing the peak areas of the peptide before and after microfiltration and the results are shown in Tables 3 and 4 below.

Table 3 - Filtration Recoveries

Formulation 0.6 mg/ml Aβ peptide in:	Filter Type (% Recoveries)		
	Millex GV	Millex GN	Millex GP
10 mM Sodium Glycinate	99.1	97.2	98.0
10 mM Arginine-HCl	91.9	86.6	92.5
10 mM Sodium Lysinate	95.0	94.7	97.4
0.01 N NH4OH	102.2	104.8	105.1

Example 4 - Filtration of Aβ42 in Sodium Glycine and Sodium Lysine Buffers With and Without Additional Excipients.

In these studies 0.6 mg/ml Aβ42 was solubilized in 10 mM sodium glycinate containing either 0.1 % polysorbate 80 (PS80), 0.9 % sodium chloride or combinations of 0.1 % PS80, 0.9 % NaCl, 5 % sucrose, 1% sucrose and/or 4 % mannitol. Approximately 2 -5 mls of each formulation was filtered through a Millex GV filter. An aliquot of the filtrate was centrifuged on a bench top micro-centrifuge set at ≥ 10,000 RPM for ~ 3 minutes. Filter recoveries were determined by comparing the RP-HPLC peak areas of the peptide before and after filtration.

Table 4 – Filtration Recoveries
For Various Buffered Formulations

Formulation ID 0.6 mg/ml Aβ42 in:	% Recovery
10 mM Sodium Glycinate, pH 9.0	104.5 %
10 mM Sodium Glycinate, pH 9.0, 0.1 % PS-80	106.2 %
10 mM Sodium Glycinate, pH 9.0, 5 % Sucrose	115.4 %

Formulation ID 0.6 mg/ml Aβ42 in:	% Recovery
10 mM Sodium Glycinate, 4 % Mannitol, pH 9.5	103.0 %
10 mM Sodium Glycinate, pH 9.5, 4 % Mannitol, 1 % Sucrose	98.0 %
10 mM Sodium Glycinate, pH 9.0, 0.9 % NaCl	76.7 %
10 mM Sodium Glycinate, pH 9.0, 0.1 % PS-80, 0.9 % NaCl	65.0 %
10 mM L-Lysine/Citrate, pH 9.5	93.2 %
10 mM L-Lysine/Citrate, 4 % Mannitol, pH 9.5	80.0 %

The Millex GV filter was established as a preferred filter for Aβ formulations owing to good recoveries of peptide and acceptability for use in commercial processes. Peptide formulations containing 0.9% NaCl were visually less soluble, leading to lower filter recoveries by RP HPLC. To increase the solubility of the peptide in the presence of inorganic salts, such as sodium chloride, it may be preferably to add a sterilized solution of the tonicity modifying agent after the microfiltration of the peptide in the buffer solution.

On the other hand the tonicity modifying agents which are saccharides (sugars) display a different type of solution activity, and may favor the maintenance of peptide suspensions. This property, known as water-ordering, tends to "hydrate" the peptide chain in solution so that it adopts its thermodynamically more stable conformation, a β-pleated sheet.

The Aβ peptide can be filtered at pH ranges from about 8.5 to about 12, preferably from pH 9 to pH 10 with good recoveries. Filtration can be accomplished with a 0.2 um Millex GV (Millipore Durapore) membrane, which is acceptable for a manufacturing process.

For a stable, biologically active formulation of Aβ peptide in a physiologically acceptable presentation suitable for use in a clinical setting, the initial step in the formulation process will preferably include a pH 8.5 to 10 sterile filtration of the peptide.

Example 5 - Soluble Liquid Formulations

Assay Methods

Three lots of Aβ42 peptide were manufactured by American Peptide Co (APC). Confirmatory formulations, chemical and biological characterization of the APC peptide results were reproduced with Aβ42 manufactured by California Peptide Research.

Formulation descriptions are as described in Parts 1, 2 and 3 below.

Stabilty Analyses.

Formulation-specific descriptions are provided in the individual sections below. Individual vials are analyzed periodically over several months storage at 2-8°C. Appearance, pH, peptide concentration and purity are routinely monitored over the course of the stability studies. A reverse phase HPLC (RP HPLC) system was used to quantitate the concentration and area percent purity of the Aβ peptide. The RP HPLC utilizes a polymeric reverse phase column, with an ammonium bicarbonate (or tris)/acetonitrile gradient elution of the peptide and detection at 220 nm. Peptide concentrations are measured against a reference standard; purity is calculated as the area percent of Aβ peptide detected in the resulting integrated chromatogram.

Characterization.

Characterization of the solution structure of Aβ42 was obtained by circular dichroism studies, as shown in Figure 1.

Biological Activity.

Swiss Webster mice (4 –8 mice per group) were injected with Aβ42 in various formulations and adjuvanted with CFA/IFA, MPL (Corixa Immunochemicals) or QS 21 (Aquila Pharmaceuticals) at antigen/adjuvant concentrations noted in the particular study. Injections were routinely administered biweekly at 0, 2, and 4 weeks unless otherwise noted. Mice were bled following the 2-week and 4-week injections. Sera were analyzed in a standard sandwich ELISA assay, utilizing Aβ42 as the antigen and HRP-conjugated goat anti-mouse IgG as the reporter antibody. Antibody titers were reported in scatter plots or as geometric means of the data for each of the animals in each group, and generally compared to titers obtained using aggregated Aβ42/CFA/IFA as the control immunogen. See Table 8 and 10 titer results.

1. Formulations.

Aβ peptide was solubilized at 0.6 mg/ml in 10 mM sodium glycinate, pH 9 – 9.5 buffer and filtered through a Millex GV 0.2 um filter. The formulation was filled at a 0.5 ml volume in 2 cc glass vials (Gensia P/N X34-113-002) and capped with gray butyl stoppers (Gensia P/N X66-113-030) and seals. Vials were stored at 2-8°C.

2. Stability Testing

The concentration and purity of Aβ42 in the formulations were monitored by RP HPLC. Soluble samples were analyzed either neat or after microcentrifugation of the samples.

The following table (Table 5) presents the analytical data for the Soluble Liquid Formulation. No difference was seen between samples which were or were not centrifuged prior to analysis, suggesting continuing solubility of the peptide at the intended concentration. The area % purity is comparable to the reference standard: no significant degradation of the peptide has been seen. The soluble formulation has remained stable over a period of 3 months when stored at 2°C to 8°C.

Table 5. Results after 3 months storage

0.6 mg/ml Aβ42 in:	Appearance	pH	Area % Purity	Aβ concentration (mg/ml)
Reference Standard	n/a	n/a	68 %	n/a
10 mM Sodium Glycinate, pH 9.0	Clear solution	8.6	69%	0.65

3. Characterization

Characterization of the Aβ formulation by circular dichroism demonstrates that the peptide assumes a random coil conformation in solution, with the characteristic negative ellipticity absorbance between 189-205 nm.

Biological Activity

The pH 9 soluble Aβ42 formulation, when injected with adjuvant, raised an antibody titer in Swiss Webster mice. Injections at biweekly intervals, (0, 2, 4 weeks) of

33 ug Aβ42 with either 50 ug MPL or 25 ug QS21 adjuvants elicited an adequate titer response in comparison to controls.

Example 6 - Liquid Suspension Formulations

Glycine/Acetate Formulations

Aβ peptide was solubilized at 0.6 mg/ml in 10 mM sodium glycinate, pH 9 – 9.5 buffer alone or with various excipients. The peptide solutions were filtered through a Millex GV filter and then titrated with 0.1M acetic acid to pH ~ 6 to create a suspension of the peptide in the buffers. The formulations were filled at a 0.5 ml volume in 2 cc glass vials (Gensia P/N X34-113-002) and capped with gray butyl stoppers (Gensia P/N X66-113-030) and seals. Formulations were stored at 2-8°C.

Glycine/Citrate Formulations

0.6 mg/ml Aβ42 formulations were prepared in 10 mM sodium glycinate containing 0.1% PS-80 alone or in combination with 5 % sucrose (25 mls). The peptide solutions were were filtered through a Millex GV filter and then titrated to approximately pH 6.0 with 0.1M citric acid. Optionally, 0.9% sodium chloride was added to the glycine/citrate/PS 80 after titration to pH 6. The formulations were then vialed at 0.5 ml fills in 2 cc glass vials (Gensia P/N X34-113-002) and capped with gray butyl stoppers (Gensia P/N X66-113-030). Formulations were stored at 2-8°C.

The Glycine/Citrate formulations were further developed to include buffering capacity at pH 6. Several 0.6 mg/ml Aβ peptide formulations at 100 ml were prepared in 10 mM sodium glycinate pH 9, containing 5% sucrose with or without 0.1% PS 80. Additional formulations at 0.1 mg/ml Aβ42 in 10 mM sodium glycinate containing 5% sucrose with or without 0.1% PS 80 were also prepared. The peptide solutions were filtered through Millex GV filters before pH adjustment. The pH was then adjusted to pH 6.0 with 10 mM and 20 mM sodium citrate buffer using a 1 M sodium citrate pH 5.5 stock solution. The formulations were then vialed at 0.5 ml fills in 2 cc glass vials (Gensia P/N X34-113-002) and capped with gray butyl stoppers (Gensia P/N X66-113-030) and aluminium seals.

Stability Testing

The concentration and purity of Aβ42 in the formulations were monitored by RP HPLC. Total peptide concentrations of the Aβ42 suspensions were measured by resolubilization of the peptide with a v:v dilution with 2 % sodium dodecyl sulfate (SDS) in 0.01 N NaOH and 1 min heating at 100°C before analysis. Alternatively, the concentration of soluble Aβ42 in the suspension formulation was determined by centrifuging the test sample in micro-centrifuge at ≥ 10,000 rpm for 3-5 minutes. Aliquots of either the resolubilized peptide or the supernatant were then analyzed by RP HPLC. Note that during the course of these studies, chromatographic improvements were made to the RP HPLC method, resulting in better resolution and quantitation. Therefore the area % purity values are relative to the reference standard at the time of analysis and chromatograms are compared at each timepoint to assess degradation. Both pH and appearance were also monitored over the course of the stability studies.

Table 6 presents the data for several liquid suspension formulations of Aβ42. All formulations are visually a suspension. These formulations are titrated to pH 6 with the respective acid listed in the table. Depending upon the excipient, a peptide suspension may occur either immediately or over a period of up to 2 weeks or more. More rapid suspension formation can be achieved by addition of sodium chloride or substitution of citrate for acetate in the peptide formulation.

All formulations achieved the target concentration of 0.6 mg/ml peptide. The area % purity for all formulations was comparable to the purity of the reference standard. No loss of peptide or degradation was seen over the 3 months storage at 2 to 8°C. The pH and appearance remained comparable to the data presented in Table 1.

Both 0.9% NaCl and 5% sucrose provide physiologic tonicity for parenteral administration. Likewise the suspension, at pH 6, is within the acceptable pH range for injection.

Table 6 Liquid Suspension Formulations

Formulation ID				mg/ml Aβ42		
0.6 mg/ml Aβ in:	Appearance	pH	Percent Purity	Total Peptide	Supernatant T=0	Supernatant T =2wks
10 mM Sodium Glycinate/Acetate, pH 6	suspension	5.9	70%	0.54	0.23	.04

10 mM Sodium Glycinate/Acetate, pH 6, 5 % Sucrose[1]	suspension	5.9	68%	0.65	0.21	.04
10 mM Sodium Glycinate/Acetate, pH 6, 0.1 % PS-80, 5 % Sucrose	suspension	6	67%	0.62	0.43	.08
10 mM Sodium Glycinate/Acetate, pH 6, 0.1 % PS-80, 0.9 % NaCl	suspension	5.9	68%	0.55	0.04	.04
10 mM Sodium Glycinate/Acetate, pH 6, 0.9 % NaCl[1]	suspension	6	70%	0.53	0.26	.05
10 mM Sodium Glycinate/Citrate, pH 6, 0.1% PS 80, 5 % Sucrose	suspension	nd	77%	0.63	<.01	nd
10 mM Sodium Glycinate/Citrate, pH 6, 0.1% PS 80, 0.9 % NaCl	suspension	nd	71%	0.63	0.02	nd

nd: not done

Table 7 formulations were prepared with either 10mM or 20mM buffering capacity provided with the citrate buffer to assure a pH of 6 is obtained during the manufacturing process. These formulations formed a suspension immediately. Due to the properties of the Aβ peptide, the change of conformation of the peptide giving a suspension occurs inside the sterile core. The use of a known buffer concentration, as opposed to titration, allows for ease of handling and reproducibility during operations within a sterile filling suite.

Table 7. Buffered Suspensions

0.6 mg/ml Aβ42 in:	Appearance	pH	Percent Purity	Total mg/ml Aβ Peptide
10 mM Sodium Citrate, 10 mM Glycine, pH 6.0, 0.1 % PS 80, 5 % Sucrose	Suspension	6.0	81%	0.66
20 mM Sodium Citrate, 10 mM Glycine, pH 6.0, 0.1 % PS 80, 5 % Sucrose	Suspension	5.9	83%	0.69
20 mM Sodium Citrate, 10 mM Glycine, pH 6.0, 5 % Sucrose	Suspension	6.0	81%	0.67
0.1 mg/ml Aβ42 in:				
20 mM Sodium Citrate, 10 mM Glycine, pH 6.0, 0.1 % PS 80, 5 % Sucrose	Suspension	5.9	82%	0.2
20 mM Sodium Citrate, 10 mM Glycine, pH 6.0, 5 % Sucrose	Suspension	6.0	81%	0.09

These formulations were further expanded. Either the triflouroacetate or the chloride salts of the Aβ42 peptide were solubilized in 10 mM sodium glycinate, pH 9 – 9.5. Concentrations were adjusted for salt content to yield a Aβ42 concentration of 0.45 mg/mL. Polysorbate 80, at concentrations ranging from 0.02% to 0.5% may be added to the peptide solutions prior to filtration. Likewise, sucrose and sodium choride may be added to the formulations, either before or after the addition of the acid. The pH of the peptide solutions was adjusted with either citrate or HCl acids, as noted below. All formulations formed suspensions, and resulted in the expected concentration of Aβ42 peptide.

Table 7b

Suspension Formulations 0.45 mg/mL Aβ42 in:
10 mM Sodium Glycinate, 20 mM Citrate, 0.1% PS-80, pH 6.0
10 mM Sodium Glycinate, 20 mM Citrate, 0.5% PS-80, pH 6.0
10 mM Sodium Glycinate, 20 mM Citrate, 154 mM NaCl, pH 6.0
10 mM Sodium Glycinate, 20 mM Citrate, 154 mM NaCl, 0.1% PS-80, pH 6.0
10 mM Sodium Glycinate, 154 mM NaCl, pH 6.0 (HCl)
10 mM Sodium Glycinate, 154 mM NaCl, 0.1% PS-80, pH 6.0 (HCl)

Characterization

Characterization of the Aβ42, pH 6 suspensions by circular dichroism and FT-IR demonstrates that the peptide assumes a beta sheet conformation as the peptide forms a suspension. The beta sheet structures are comparable in the 0.6 mg/ml suspensions, regardless of the buffering acid (citrate, acetate, phosophate) or additional excipients (sucrose, NaCl) added to the formulations. The addition of PS-80 appears to generate a more uniformly dispersed suspension.

Biological Activity

The Aβ peptide suspension, when injected with adjuvant, raises an antibody titer in Swiss Webster mice. Injections at biweekly intervals, (0, 2, 4 weeks) as shown in Table 8 elicited an adequate titer response in comparison to the control.

Table 8. Antibody Response to Suspension Formulations

Peptide Formulation 0.6 mg/ml Aβ in:	ug Peptide	ug Adjuvant	Antibody Titers Geometric means 2nd bleed**
10 mM Sodium Glycinate/Acetate, pH 6, 5 % Sucrose	33 ug	50 ug MPL*	≈ 7000
10 mM Sodium Glycinate/Acetate, pH 6, 5 % Sucrose	33 ug	25 ug QS 21	≈ 10,000
10 mM Sodium Glycinate/Acetate, pH 6, 0.1 % PS-80, 5 % Sucrose	33 ug	50 ug MPL*	≈ 10,000
10 mM Sodium Glycinate/Acetate, pH 6, 0.1 % PS-80, 5 % Sucrose	33 ug	25 ug QS 21	≈ 7000
10 mM Sodium Glycinate/Citrate, pH 6, 0.1% PS 80, 5 % Sucrose	33 ug	50 ug MPL*	≈ 10,000
10 mM Sodium Glycinate/Citrate, pH 6, 0.1% PS 80, 5 % Sucrose	33 ug	25 ug QS 21	≈ 8,000
10 mM Sodium Glycinate/Citrate, pH 6, 0.9 % NaCl	33 ug	50 ug MPL*	≈ 12,000
10 mM Sodium Glycinate/Citrate, pH 6, 0.9 % NaCl	33 ug	25 ug QS 21	≈ 14,000
Control: Calif. Peptide lot MF0639	33 ug	CFA/IFA	≈ 1,200

* MPL formulation containing triethanolamine

** Titers calculated as units at 50% max. OD

Example 7 - Lyophilized Formulations

Formulations.

Four combinations of 0.6 mg/ml Aβ42 in 10 mM glycinate or lysine buffer with mannitol or mannitol and sucrose were prepared and sterile filtered through a Millex GV filter. One formulation, 0.6 mg/ml Aβ42 in 10 mM sodium glycinate pH 9.5, 4% mannitol, was vialed and stoppered without titration to a physiological pH. The remaining three solutions (lysine/citrate/4% mannitol; glycinate/citrate/4% mannitol; glycinate/HCl/4% mannitol/1% sucrose) were then titrated to pH 7.5 with either citric acid or HCl as noted. The formulations were then vialed at 0.5 ml fills in 2 cc glass vials

(Gensia P/N X34-113-002) and loosely capped with grey butyl lyophilization stoppers (Gensia P/N X66-113-030).

Lyophilization.

The formulations were lyophilized in a programmable Virtis lyophilizer (provided by Gensia Sicor). Mannitol was chosen as the primary matrix component. To achieve crystallization of the mannitol, the formulations were frozen with thermal cycling (annealing), followed by primary and secondary drying of the cakes.

Stability Analyses.

The concentration and purity of the lyophilized formulations was monitored by RP HPLC. Each formulation was reconstituted with 1.0 ml water for injection (WFI). Formulations were analyzed immediately after reconstitution (with or without centrifugation in a bench top micro-centrifuge at ≥ 10, 000 rpm for 3-5 minutes) or resolubilized as described previously.

The following table (Table 9) tabulates the concentration of the four Aβ42 formulations following lyophilization and reconstitution. As can be seen from the data, these formulations were soluble. Samples were analyzed both as the reconstituted formulation, and as the reconstituted formulation "resolubilized" as described for the peptide suspensions. No significant difference was observed in the reconstituted formulation versus the total peptide concentration (resolubilized sample). No significant degradation or loss in peptide was seen over the course of 3 months storage at 2 to 8 °C as determined by RP HPLC as in the stability testing for Example 5.

Table 9. Lyophilized formulations

Formulation 0.6 mg/ml Aβ42 in:	Appearance	pH	% Purity	mg/ml Aβ42	
				Reconstituted peptide	Resolubilized peptide
10 mM Sodium Glycinate, pH 9.5, 4 % Mannitol	Clear solution	8.2	82%	0.59	0.61
10 mM Sodium Glycinate/citrate, pH 7.5, 4 % Mannitol	Clear solution	7.4	82%	0.57	0.60
10 mM Sodium Lysinate/citrate, pH 7.5, 4 % Mannitol	Clear solution	7.6	81%	0.57	0.55

10 mM Sodium Glycinate/HCL, pH 7.5, 4 % Mannitol, 1 % sucrose	Clear solution	7.4	81%	0.56	0.59

Characterization

Characterization of the Aβ42 pH 7.5 lyophilized formulations by circular dichroism and FTIR demonstrates that the peptide remains in a random coil conformation throughout lyophilization and reconstitution. This observation is consistent with the Aβ42 sodium glycinate pH 9 formulation which is also soluble, filterable and assumes a random coil conformation in solution.

Biological Activity

The Aβ42 lyophilized formulation, 0.6 mg/ml Aβ42 in 10mM sodium glycinate/HCl/4% mannitol/1% sucrose, pH 7.5, was chosen as a representative lyophilized formulation. This product, when mixed with either MPL or QS21 adjuvant, raises an antibody titer in Swiss Webster mice. Geometric mean titers are shown in Table 10 in comparison to controls.

Table 10 Antibody Response to Lyophilized Preparations

Peptide Formulation 0.6 mg/ml Aβ42 in:	ug Peptide	ug Adjuvant	Antibody Titers Geometric means 2nd bleed**
10 mM Sodium Glycinate/HCL, pH 7.5, 4 % Mannitol, 1 % sucrose	33 ug	50 ug MPL*	≈ 14,000
10 mM Sodium Glycinate/HCL, pH 7.5, 4 % Mannitol, 1 % sucrose	33 ug	25 ug QS 21	≈ 3,000
Control: Calif. Peptide lot MF0639	33 ug	CFA/IFA	≈ 1,200

*MPL formulation containing triethanolamine
**Titers calculated as units at 50% max. OD

Example 8 - Scale-up using GMP manufacturing standards

The peptide suspension was scaled up to a 1.5-liter scale, manufactured and filled at a contract GMP drug product manufacturer. Two concentrations were filled: 0.6

mg/ml and 0.1 mg/ml. Both concentrations were successfully scaled up, filled, and remain stable at 2 – 8°C and at 25°C after two months.

Aβ peptide was dissolved at either 0.6 mg/ml or 0.1 mg/ml concentrations in a 10 mM sodium glycinate pH 9 buffer containing 5% sucrose. The peptide solution was sterile filtered through a Millipak 20 Millipore Durapore 0.2 um sterilizing filter, into the aseptic core. RP HPLC analysis demonstrated no significant loss of peptide throughout the filtration process. A 1 M sodium citrate pH 5.5 buffer was compounded, and likewise sterile filtered through a 0.2 um Durapore filter into the aseptic core. The peptide solution was weighed, and an appropriate amount of sodium citrate buffer added to yield a 20 mM citrate, pH 6 formulation.

At the 0.6 mg/ml concentration, the peptide immediately formed a suspension upon addition of the citrate buffer; the in-process pH measurement was 6.4. The peptide suspension was constantly stirred, filled at 1.2 ml per vial into 2cc borosilicate glass vials, and sealed with West 4416 stoppers and seals. The 0.1 mg/ml concentration remained soluble (and was filled soluble) for several hours before forming a suspension in the vials by the next day.

Table 11 presents the data generated for this 0.6 mg/ml Aβ42 suspension:

Table 11

Aβ Peptide
0.6 mg/ml suspension
in 10 mM glycine, 20 mM citrate, 5% sucrose, pH 6

Test	Specification	Result
Appearance	Hazy colorless suspension, substantially free of contaminant particles	Pass
PH	5.5-6.5	6.3
Concentration	0.5 – 0.7 mg/ml	0.64 mg/ml
Area Percent Purity (HPLC)	FIO	84.4%
Volume, ml	FIO	1.13 ml
Bulk Sterility	FIO	No growth
Final Container Sterility	No growth	Pass
Bacterial endotoxins	FIO	< 2 EU/ml

Example 9

Buffered suspensions of Aβ1–42 with QS-1 adjuvant

Single vial formulations incorporating Aβ1-42 and an adjuvant were studied using QS-21, a triterpene glycoside having immune stimulating activity, and surprisingly found to result in the formation of a visually clear suspension of the peptide.

1. Single Vial Formulation of Aβ1-42 /QS-21

Solubilization of lyophilized QS-21 with Aβ1-42 solution in 10 mM sodium glycinate at pH 9.0. In each of the following examples, lyophilized QS-21 was solubilized with A□1-42 (TFA salt, 0.45 mg/mL) solution in 10 mM Glycine (Gly), pH 9.0. The pH of the composition containing the solubilized QS-21 was then rapidly adjusted to 6.0 by the addition of citrate buffer (Cit) to form a visually clear suspension. Turbidity measurements were determined with a spectrophotometer set at a wavelength of 405 nm to measure the clarity of the resulting suspensions. The results indicate that the particle size of the suspended peptide is smaller than the wavelength of the reflected light. These formulations have demonstrated 3 months stability with storage at 2-8°C with ongoing stability monitoring; all formulations were found to contain Aβ1-42 predominantly in the β-sheet conformation; all formulations showed no turbidity when analyzed in a spectrophotometer at 405 nm. The 0.45 mg/mL Aβ1-42 , 0.2 mg/mL QS-21, 10 mM Gly, 20 mM Cit, 5 % Sucrose, pH 6.0 formulation was tested in the mouse titer assay, resulting in a high titer response.

Table 12A

Formulation	Appearance
0.45 mg/mL Aβ1-42 , 0.2 mg/mL QS-21, 10 mM Gly, 20 mM Cit, 5 % Sucrose, pH 6.0[1]	Clear
0.45 mg/mL Aβ1-42 , 0.1 mg/mL QS-21, 10 mM Gly, 20 mM Cit, 5 % Sucrose, pH 6.0	Clear
0.225 mg/mL Aβ1-42 , 0.3 mg/mL QS-21, 10 mM Gly, 20 mM Cit, 5 % Sucrose, pH 6.0	Clear
0.225 mg/mL Aβ1-42 , 0.15 mg/mL QS-21, 10 mM Gly, 20 mM Cit, 5 % Sucrose, pH 6.0	Clear

[1]Found to have high mouse titer response

2. Aβ42 Titrations with QS-21

Aβ42 (2 mg/mL) was solubilized in 10 mM Glycine, pH 9.5. The pH was readjusted to 9.5 with 1 N NaOH. The Aβ42 solution was then filtered through a Millex GV filter. QS-21 (5 mg/mL) was solubilized in 10 mM Citrate, pH 6.0 and was then filtered through Millex GV filter. Aliquots of Aβ42 were mixed with aliquots of QS-21 to give the desired ratio, mixed, and diluted with 10 mM Glycine, pH 9.5 and 1 M Citrate, pH 5.2, to yield the final concentrations noted in Table 12 below.

Table 12B. Initial AN1792/QS21 Co-Formulations

Formulations In 10 mM Glycine, 20 mM Citrate, pH 6.0	Appearance	$OD_{405\ nm}$
1.0 mg/mL Aβ42, 1.0 mg/mL QS-21	clear	0.018
1.0 mg/mL Aβ42, 0.5 mg/mL QS-21	opaque (+)	0.013
1.0 mg/mL Aβ42, 0.25 mg/mL QS-21	opaque (++)	0.030
1.0 mg/mL Aβ42, 0.125 mg/mL QS-21	opaque (+++)	0.175
1.0 mg/mL Aβ42, 0.063 mg/mL QS-21	opaque (+++)	0.308
1.0 mg/mL Aβ42, 0.031 mg/mL QS-21	opaque (+++)	0.315
1.0 mg/mL Aβ42, 0.016 mg/mL QS-21	opaque (+++)	0.268
1.0 mg/mL Aβ42, 0.0 mg/mL QS-21	opaque (+++)	0.213
0.5 mg/mL Aβ42, 1.0 mg/mL QS-21	clear	0.005
0.5 mg/mL Aβ42, 0.5 mg/mL QS-21	clear	0.006
0.5 mg/mL Aβ42, 0.25 mg/mL QS-21	clear	0.005
0.5 mg/mL Aβ42, 0.125 mg/mL QS-21	opaque (+)	0.021
0.5 mg/mL Aβ42, 0.063 mg/mL QS-21	opaque (++)	0.148
0.5 mg/mL Aβ42, 0.031 mg/mL QS-21	opaque (+++)	0.172
0.5 mg/mL Aβ42, 0.016 mg/mL QS-21	opaque (+++)	0.162
0.5 mg/mL Aβ42, 0.0 mg/mL QS-21	N/A	N/A
0.3 mg/mL Aβ42, 1.0 mg/mL QS-21	clear	0.004
0.3 mg/mL Aβ42, 0.5 mg/mL QS-21	clear	0.004
0.3 mg/mL Aβ42, 0.25 mg/mL QS-21	clear	0.006
0.3 mg/mL Aβ42, 0.125 mg/mL QS-21	clear	0.005
0.3 mg/mL Aβ42, 0.063 mg/mL QS-21	opaque (+)	0.013
0.3 mg/mL Aβ42, 0.031 mg/mL QS-21	opaque (+)	0.077
0.3 mg/mL Aβ42, 0.016 mg/mL QS-21	opaque (+)	0.068
0.3 mg/mL Aβ42, 0.0 mg/mL QS-21	opaque (++)	0.046
0.1 mg/mL Aβ42, 1.0 mg/mL QS-21	clear	0.004
0.1 mg/mL Aβ42, 0.5 mg/mL QS-21	clear	0.002
0.1 mg/mL Aβ42, 0.25 mg/mL QS-21	clear	0.002

0.1 mg/mL Aβ42, 0.125 mg/mL QS-21	clear	0.001
0.1 mg/mL Aβ42, 0.063 mg/mL QS-21	clear	0.002
0.1 mg/mL Aβ42, 0.031 mg/mL QS-21	clear	0.003
0.1 mg/mL Aβ42, 0.016 mg/mL QS-21	clear	0.004
0.1 mg/mL Aβ42, 0.0 mg/mL QS-21	clear	0.008

3. Additional formulations of Aβ42 and QS-21

Aβ42, chloride salt, was solubilized atl mg/mLin 10 sodium glycinate, , pH 9.0 - 9.5 with or without 5 % sucrose, 0.1 % PS-80, or 0.4 % PS-80. Peptide solutions were sterile filtered through Millex GV syringe filters. Lyophilized QS-21 was solubilized at 5 mg/mL in 10 mM citrate, pH 6.0, and sterile filtered through a Millex GV syringe filter.

Appropriate volumes of the Aβ42 solutions and the QS-21 solutions were combined to yield the final concentrations of Aβ42 and QS-21 noted in the formulations in Table XX below. Last, the pH was lowered with 1M Citrate buffer, pH 5.4, to give a final pH of 6 in 20 mM citrate buffer. The visual appearance of the formulations was rated as clear to cloudy (+ to +++). Varying the concentrations and ratios of Aβ42 and QS-21 can moderate the visual appearance of the formulations. Furthermore, sugars and surfactants are acceptable additional excipients to the formulations.

Table 12C. Additional Formulations of Aβ42 and QS-21

Formulations			Appearance			
QS-21 (mg/mL)	Aβ42 (mg/mL)	Aβ42/ QS-21 Ratio	No Excipient	5 % Sucrose	0.1 % PS-80	0.4 % PS-80
0.05	0.05	1.0	Clear	Clear	Clear	Clear
0.1	0.1	1.0	Clear	Clear	Clear	Clear
0.2	0.2	1.0	Clear	Clear	Clear	Cloudy (+)
0.1	0.23	2.3	Cloudy (+)	Cloudy (+)	Cloudy (+)	Cloudy (+)
0.2	0.23	1.1	Clear	Clear	Clear	Cloudy (+)
0.3	0.23	0.8	Clear	Clear	Clear	Cloudy (+)
0.1	0.45	4.5	Cloudy (++)	Cloudy (++)	Clear	Cloudy (++)
0.2	0.45	2.3	Cloudy (++)	Cloudy (++)	Cloudy (+)	Cloudy (++)
0.3	0.45	1.5	Clear	Clear	Clear	Cloudy (++)

Example 10 - Measurement and Interpretation of C.D. Spectra of Aβ42 Formulations

The circular dichroism data were collected using an Aviv model 62-DS spectropolarimeter (Lakewood, NJ). Samples were prepared appropiately for collection of near UV and far UV spectra, respectively, and loaded into a strain-free 1 mm pathlength quartz cell. The sample holder was held at a steady temperature of 25° C. Data were collected at 0.5 nm intervals using a 4 second averaging time. In the far UV region ($\lambda \sim$ 180-250 nm), signals from the peptide backbone dominate the spectrum and estimates of the secondary structure composition can be obtained. Examination of the near UV ($\lambda \sim$ *1250-350* nm) region provides quite different information: in this region the primary signals arise from aromatic side chains (Phe, Tyr, and Trp). The sign and magnitude of the signal indicates the degree of flexibility at each site as well as the orientation of the side chain relative to the peptide backbone. As the number of these chromophores is less than the number of amide groups, and because the chromophores are distributed throughout the molecule, they provide some indication of local structure.

Numerous modifications and variations in the invention as described in the above illustrative examples are expected to occur to those skilled in the art and, consequently, only such limitations as appear in the appended claims should be placed thereon. Accordingly, it is intended in the appended claims to cover all such equivalent variations, which come within the scope of the invention as claimed.



We claim:

1. A composition comprising an aqueous solution of at least 0.01 mg/ml of Aβ peptide wherein said aqueous solution is maintained at a pH sufficient to solubilize said Aβ peptide.

2. The composition of claim 1, wherein the solution is maintained at such a suitable pH by use of an effective amount of a pharmaceutically acceptable buffer.

3. A composition comprising a sterile aqueous solution comprising at least 0.01 mg/ml of Aβ peptide wherein said aqueous solution is maintained at a pH sufficient to solubilize said Aβ peptide.

4. The composition of claim 3 wherein the solution is maintained at such a pH by use of an effective amount of a pharmaceutically acceptable buffer.

5. The composition of claims 1 or 3, wherein said Aβ peptide is a long form of Aβ peptide.

6. The composition of claims 1 or 3, wherein said Aβ peptide is Aβ42.

7. The composition of claims 1 or 3, wherein the pH is about 8.5 to about 12.

8. The composition of claim 7, wherein the pH is about 9 to about 10.

9. The composition of claims 2 or 4, wherein the pharmaceutically acceptable buffer is selected from the group consisting of amino acids, salts and derivatives thereof; pharmaceutically acceptable alkalizers, alkali metal hydroxides and ammonium hydroxides, organic and inorganic acids and salts thereof; and mixtures thereof.

10. The composition of claim 9 wherein the pharmaceutically acceptable buffer is glycine (sodium glycinate) or arginine (arginine hydrochloride).

11. A lyophilized composition of Aβ peptide which composition is prepared by the process of:

a) freezing a sterile aqueous solution having at least 0.01 mg/ml of Aβ peptide wherein said aqueous solution is maintained at a pH sufficient to solubilize said Aβ peptide; and

b) lyophilizing the frozen composition prepared in a) above.

12. The composition of claim 11, wherein said Aβ peptide is a long form of Aβ peptide.

13. The composition of claim 11, wherein said Aβ peptide is Aβ42.



14. The composition of claim 11, wherein the solution is maintained at such a pH by use of an effective amount of a pharmaceutically acceptable buffer.

15. The composition of claim 14, wherein the pharmaceutically acceptable buffer is selected from the group consisting of amino acids, salts and derivatives thereof; pharmaceutically acceptable alkalizers, alkali metal hydroxides and ammonium hydroxides, organic and inorganic acids and salts thereof; and mixtures thereof.

16. The composition of claims 1, 3 or 11, wherein the Aβ peptide is substantially in a random coil conformation.

17. The composition of claims 1, 3 or 11, wherein the Aβ has a concentration of from about 0.05 mg/ml to about 2.0 mg/ml.

18. The composition of claims 1, 3 or 11, wherein the composition further comprises a pharmaceutically acceptable adjuvant.

19. The composition of claim 18, wherein the adjuvant is selected from the group consisting of incomplete Freund's adjuvant; MPL; QS-21; and alum.

20. A composition comprising a sterile aqueous peptide suspension of at least 0.1 mg/ml of Aβ peptide at a pH of about 5 to about 7.

21. The composition of claim 20 wherein the aqueous peptide suspension also contains an effective amount of a pharmaceutically acceptable buffer.

22. The composition of claims 20 or 21 wherein said Aβ is a long form of Aβ peptide.



23. The composition of claim 22 wherein said Aβ peptide is Aβ42.

24. The composition of claim 21 wherein the pharmaceutically acceptable buffer is selected from the group consisting of amino acids, salts and derivatives thereof; pharmaceutically acceptable alkalizers, alkali metal hydroxides and ammonium hydroxides, organic and inorganic acids and salts thereof; and mixtures thereof.

25. The composition of claim 20 having 0.1 to 0.8mg/ml of Aβ42 peptide, 10 mM glycine, and an acid sufficient to adjust the pH to about 5.5 to about 6.5.

26. The composition of claims 24 or 25 further comprising one or more excipients chosen from the group consisting of tonicity modifiers, surfactants, and wetting agents.

27. The composition of claim 24 wherein the composition further comprises a pharmaceutically acceptable adjuvant.

28. The composition of claim 26 wherein the composition further comprises a pharmaceutically acceptable adjuvant.

29. The composition of claim 28 wherein the adjuvant is selected from the group consisting of incomplete Freund's adjuvant; MPL; QS-21 and alum.

30. The composition of claim 28 having about 0.1 to about 1.0 mg/ml of Aβ42 peptide in 10 mM glycine, and at least 0.1 mg/ml of QS-21 in an amount effective to form a visually clear suspension, having a pH of about 6.

31. A process for preparing a sterile composition of a long form of Aβ peptide comprising:

adjusting the pH of an aqueous solution sufficient to solubilize the Aβ peptide therein;

dissolving into the solution an amount of the Aβ peptide sufficient to achieve an immunogenic concentration for a mammal; and

filtering the resulting solution through a uniform pore size membrane said pore size being in a range capable of excluding bacteria and passing substantially all of the Aβ peptide through the membrane.

32. The process of claim 31 wherein the filtration is effected with a hydrophilic polymer membrane having a uniform pore size of about 0.22 microns.

33. The process of claim 31, wherein the amount of Aβ peptide recovered after filtration is greater than 50%.

34. The process of claim 31, wherein the prefiltration solution contains at least one diluent chosen from the group consisting of pharmaceutically acceptable buffers having a concentration of from about 5 mM to about 45 mM.

35. The process of claim 34, wherein the prefiltration solution contains a tonicity modifying agent from about 0.9% to about 6.0%(w/v).

36. The process of claim 34, wherein the prefiltration solution contains a surfactant from about 0.02 to about 1.0 % (w/v).

37. The process of claim 34, wherein the prefiltration solution contains a chelating agent from about 0.1mM to about 1.0 mM.

38. The process of claims 34, 35, 36 or 37 wherein the pH of the sterile solution resulting after filtration is adjusted to pH about 5 to about 7 to provide a peptide suspension.

39. A method for preventing or treating Alzheimer's disease in a mammal comprising administering to said mammal a sufficient amount of a sterile aqueous composition comprising at least 0.05 mg/ml of Aβ peptide to induce an immunogenic response in said mammal wherein said aqueous solution is maintained at a pH sufficient to solubilize said Aβ peptide.

40. A method of invoking antibody response against an Aβ peptide in a mammal in need of such an antigenic response comprising:

parenterally administering an immunogenic amount of a sterile composition of a long form of Aβ.



41. The method of claims 39 or 40, wherein the method further comprises administering a pharmaceutically acceptable adjuvant separately or admixed in within the said sterile composition.

42. The method of claims 39 or 40, wherein the sterile composition is according to claim 30.

43. A composition comprising a suspension of at least 0.1 mg/ml Aβ peptide and an effective amount of QS-21 to form a visually clear suspension in the pH range of 5 to 7.

44. A composition comprising a suspension of at least 0.1 mg/ml Aβ peptide and an effective amount of DPPC(dipalmitoyl phosphatidyl chloride) to form a visually clear suspension in the pH range of 5 to 7.

45. Use of a sterile composition of a long form of Aβ for the manufacture of a medicament for invoking antibody response against an Aβ peptide.

46. Use of a sterile aqueous composition of Aβ peptide for the manufacture of a medicament useful for preventing or treating Alzheimer's disease.

47. Use of claim 45 or 46 wherein said medicament further comprising a pharmaceutically acceptable adjuvant.

This Sheet Has Been Replaced

