

FULLERENE GABA DOPA AND METHODS

BACKGROUND

5 1. FIELD OF INVENTION

[0001] The present invention is a composition of buckminsterfullerene with gamma amino butyric acid (GABA) and levodopa (L-dopa) or dopamine (DOPA) pendant groups that can function as a dual neurotransmitter, and methods of use to prevent or to treat degenerative neural disease and loss of motor neuron control that is associated with synaptic damage or neural cell
10 death in susceptible cells. Delivery methods include ingestion, inhalation, or injection when used as a medicament or as a food supplement to maintain or re-establish benign healthy neural cellular homeostasis.

2. BACKGROUND ART

[0002] Parkinson's disease (PD) is a movement disorder driven by the loss of dopamine producing neurons in the substantia nigra (SN) region of the human brain. PD is characterized by difficulty in initiating movement, muscle rigidity, muscle tremors, and an inability to maintain a stable posture. The motor dysfunctions represent the major clinical features of this disease. Non-motor symptoms such as sleep disturbances, dementia and depression may also be present. Motor disturbances are primarily produced by the degeneration of dopamine neurons in the SN, as well as
15 the projections from this region to the striatum. Additional regions of neurons may also be affected in the disease. One short term treatment strategy was based on prescribing dopamine neurotransmitter agonists. However, it was found that even when administered in combination with dietary antioxidants, dopamine promoters and dopamine itself can be ineffective or even produce negative effects after long-term administration.

25 [0003] Decreased levels of total glutathione (GSH) and oxidized glutathione (GSSG) with 40% to 50% deficit were found in the substantia nigra region of the autopsied brains from deceased patients with PD by Perry and colleagues in the 1980's. Later, a similar loss of antioxidant GSH in the substantia nigra was found in Incidental Lewy body disease. Lewy body disease is thought to be an early form of PD. In these cases, oxidative damage to lipids, protein, and DNA in the nigra of PD
30 patients occurs. Past clinical trials attempting to treat PD have used antioxidants targeted to substitute for GSH, but these substances have shown little benefit, likely because of low bioavailability to the brain.

[0004] Identification of the oxidative properties of dopamine implicated it as one potential source of oxidative stress in PD when it is present under oxidative conditions. The discovery of PD-causing mutations causing alpha-synuclein aggregation in the Incidental Lewy body pathology of PD leading to neurodegeneration was later clarified by manipulation of both dopamine levels and α -

5 synuclein expression in aged mice. It was then found that only the combination of these 2 factors caused progressive neurodegeneration of the SN region of the brain and an associated motor deficit.

[0005] Alpha synuclein in fibril form is a 14 kilo-Dalton protein. The neurodegenerative disorders associated with alpha-synuclein plaques are collectively known as synucleinopathies.

The major components of intraneuronal inclusions containing alpha-synuclein plaques are termed

10 Lewy bodies. It is now well understood that the key symptoms of PD and Lewy body disease is the aggregation of alpha-synuclein protein fibrils into pathogenic alpha-synuclein plaques. Such toxic plaques act as a catalyst to agglomerate and recruit healthy independent alpha-synuclein fibrils into greater numbers of oligomeric toxic plaques. The alpha-synuclein plaques and other

15 oligomeric particles that are implicated in amyotrophic lateral sclerosis can be considered a type of prion, although these plaques may be better termed idiopathic or systemic prions and are likely not infectious between individuals. However, toxic oligomeric plaques are quite capable of migrating along the neurons to affect the neural structures in the gut, the vagus nerve, and anywhere that neurotransmitters such as dopamine can be exchanged between synapses of different

20 neural cells.

[0006] Genetic point mutations were found to cause autosomal forms of PD by pathogenic disruption of the amino acids in the hydrophobic N-terminal domain of alpha-synuclein to cause nucleation and aggregation into β -sheet-rich oligomeric plaques. Under some oxidative conditions, the oligomer attaches to the neurotransmitter dopamine and can migrate from neuron to neuron and

25 thereby modify alpha-synuclein aggregation in the SN, resulting in greater abundance of alpha-synuclein oligomers and dopamine-induced plaques. Furthermore, it was found that by disrupting the dopamine- α -synuclein interaction under these conditions, dopaminergic neuronal degeneration was reversed. Equally important and extensive evidence from cultured cells and cell-free solution testing indicates that the neurotransmitter dopamine may, under reducing conditions, reverse or inhibit the

30 aggregation of alpha-synuclein. At present, L-dopa, a dietary supplement that metabolizes into dopamine neurotransmitter, is the most widely used and most effective therapy for Parkinson's disease (PD).

[0007] Elevating dopamine levels under either oxidizing or reducing conditions either increased or reduced the number of cells containing insoluble and large intracellular alpha-synuclein plaques. This demonstrated that the interaction of dopamine and alpha-synuclein is noncovalent and reversible, and it also demonstrated that reduction-oxidation (REDOX) conditions reversed the conditions associated with PD. It is historically and medically significant to understand that oxidized dopamine promotes the formation of alpha-synuclein plaques that become neurotoxic in large concentrations. This pathology is caused by disrupting cytoskeletal organization in neural lipid membranes that reduce synaptic terminal membrane permeability and affects neural signaling by blocking calcium ion transport.

[0008] In more detail, it is now clear that healthy individual alpha-synuclein fibrils in neurons bond with the inner (cytosolic) leaflets of the presynaptic membrane leaflet to induce a concentration dependent stabilization of docked and double-anchored synaptic vesicles. Neurodegenerative oxidative and free radical damage forms cross-links within and thickens the overall lipid composition of the cellular membranes. This chemical change allows alpha-synuclein to bond to outer presynaptic membranes and disrupts the correct morphology and conformation of alpha-synuclein, encouraging the formation of plaques. The movement of toxic plaques to the inner presynaptic membranes then causes dysfunctional disruption of synaptic vesicle membrane transport.

[0009] On a related topic, glutathione (GSH) is the most abundant small molecule, non-protein thiol in cells that acts as a reducing agent. It consists of a tripeptide of glutamate, cysteine, and glycine. Within neurons, GSH is found in greatest abundance at nerve endings. It is now well understood that GSH in the neural synapse serves as a REDOX regulator that functions to control and maintain intracellular REDOX. Moreover, the absence of reductive GSH in the substantia nigra region of the brain leads to dysregulated conditions that promote neurotoxic alpha-synuclein plaque formation. The degeneration of dopaminergic neurons in the substantia nigra during PD is therefore directly related to GSH depletion that leads to elevated levels of nitric oxide and peroxynitrite oxidants, leading to oxidative stress damage. This damage inhibits the REDOX complexes of the electron transport chain, causes a drop in the proton motive force, and reduces ATP production to further magnify the REDOX dysfunction by causing mitochondria to significantly reduce GABA (GSH) synthesis. The further loss of GSH increases oxidative and free radical damage to surrounding cellular lipids and increasingly creates those toxic conditions leading to neurodegeneration.

[0010] Given these understandings of the etiology and neuronal disease characteristics associated with the progression of PD, designs of materials or compositions able to decelerate or alleviate PD and related motor neuron disease progression is now better focused, and the targets of oxidative stress and plaque formation have achieved great importance. In particular, the anti-aggregation effect of fullerenols, previously demonstrated for various forms of amyloid proteins, was also observed to be somewhat effective for the reduction of alpha-synuclein insoluble protein aggregates. The limited fullereneol neuroprotective activity was not affected by the number of carbon atoms in the fullereneol compounds, so that polyhydroxylated C60 or C70 was able to act equally well. While fullerenols might be a promising tool for drug development to treat PD, these materials are also understood to be insufficiently targeted to the specific regions of neuronal dysfunction, such as the SN region of the PD brain, and do not have an established or significant ability to cure or prevent PD.

[0011] The consensus among scientists and medical practitioners is that oxidative stress plays a major role in the development of neural cellular metabolic disease. Some of the current therapeutic strategies have put emphasis on the design of multiple functional properties into molecules or particles that enable them to target multiple enzymes or receptors, allowing them all to operate at the same time to avoid or correct the dysfunctions leading to disease states. Of these, several natural antioxidants have been known for their ability to trap and scavenge free radical species and reactive oxygen species (ROS) such as hydroxyl and nitrate free radicals. ROS are associated with causing damage to essential cellular components, and neurodegenerative diseases.

[0012] While there is great promise in the widely known beneficial neuroprotective functions of gamma-aminobutyric acid (GABA) in the brain, there are no known strategies to enhance its poor oral bioavailability to deliver this substance to the substantia nigra portion of the human brain. This is because it is widely believed that one of the precursor glutamine or glutamine amino acids already penetrate the blood-brain barrier, and therefore the methionine cycle will work to generate GABA endogenously and locally. However, it is also verified that the endogenous production of certain substances like glutathione has failed locally in the Parkinson's brain. It happens that glutathione is also involved in the methionine cycle. Thus, the medical evidence for sufficient endogenous GABA production in all brain tissues remains an assumption or a hypothesis not yet proven or disproven by conclusion. This type of investigation is important because GABA is the principal inhibitory neurotransmitter in the mammalian central nervous system. Dysregulation of GABA is already

implicated in many neurological disorders, such as in Alzheimer's disease, epilepsy, panic disorder, and anxiety.

[0013] GABA is what is called a zwitterion. In this nature of GABA, the amine and the carboxylic acid groups on this molecule are both protonated and deprotonated, respectively, at physiological pH. Endogenously produced GABA is a neurotransmitter that has outstanding benefits, including reducing blood pressure, enhancing long-term memory, and improving diabetes by controlling the secretion of insulin. However, GABA is easily broken down by neural enzymes, especially as released by astrocytes. This technological hurdle may help to explain why a possible beneficial effect of GABA into the brain to treat Parkinson's disease has never been studied or seriously practiced in medical neuroscience.

[0014] Many proposed treatments or compositions in clinical trials have proven insufficiently bioavailable, being poorly soluble in water or being unable to pass cellular membranes. Typical drug loading of about 10 % is achieved for nanoparticles of a narrow size distribution around an average size of about 100 nanometers when encapsulated in water soluble polymer micelles. While this method suggests complete dispersibility of drug substances, the dispersion bonding is often substantially irreversible, and may lead to significant masking of the therapeutic agent, along with poor targeting to the appropriate molecular receptors and affected structural regions of neural cells. The dual bioavailability and specific targeting problems remain significant obstacles to any significant commercial and medical success of multifunctional prophylactic protective and therapeutic neurological compositions for alpha-synuclein or dopamine dominated neural dysfunction and neurological disease.

[0015] Dopamine agonists are state of the art medications based on a derivative of dopamine that have been medically proven to stimulate the parts of the human brain influenced by dopamine. The neurons of the brain, especially in the substantia nigra where motor control is interfaced with control signals propagating into and from the brain stem, can accept these exogenous, artificial, and introduced dopamine substitutes. The neurons are then able to perform and function as if accepting the endogenous dopamine that these neurons need but have not been able to receive in sufficient quantity over the early stages of the neurological disease. In general, dopamine agonists are not as potent as carbidopa or levodopa and may be less likely to cause dyskinesias. However, a better solution is not commercialized yet, and most people who take levodopa develop motor control problems within 5 to 10 years after starting their dopamine agonist medicine. These complications are unpredictable swings in muscular position control between

doses and uncontrollable jerking or twitching (dyskinesias). Dyskinesia can become so intense that that it is as disabling as some of the problems caused by the neurological disease. In the longest study done, people who started treatment with a dopamine agonist had just as many problems with motor fluctuations at 14 years as people who started treatment with levodopa, indicating a failure to prevent oxidative damage at the post synaptic bouton, a failure to protect neurons from cell death, and a failure to prevent the accumulation of toxic brain protein oligomers such as alpha-synuclein plaques that are a cause of neural cell death. The study of Olanow (2013) published that those observed factors most predictive of developing dyskinesia from L-dopa and agonists of dopamine in combination with L-dopa. These predictive results are ranked order from most to least, being: (1) young age at onset, (2) higher L-dopa dose, (3) low body weight, (3) North American geographic region, (4) The L-dopa with carbidopa and entacapone (two agonist drugs) treatment group, (5) female gender, and (6) a more severe Unified Parkinson's Disease Rating Scale (UPDRS) Part II. There is clear medical benefit, medically verified neuroprotective effects, and no known toxicity of L-dopa administered either at early stages or at late stages of Parkinsonism. However, there can also be side effects from L-dopa treatment either in combination with or without dopamine agonists: these side effects may include gastrointestinal disturbances, cognitive problems, and sedation. The more recently approved combination therapies of L-dopa with the newer dopamine agonist drugs (also known as dopaminergic substances) still cause dyskinesia in late-stage Parkinson's disease, where this problem persists to the modern day, in the state of the art; a study by Shiraishi (2019) is one such example.

[0016] The severe economic impact to families and society from insufficiently comprehensive state-of-the-art neural disease treatments for conditions such as Parkinson's disease remains a burden that society can ill afford. Moreover, the advent of COVID-19 and mutated novel coronavirus has led to a rise in the incidence of Parkinsonism in many affected individuals.

[0017] What is therefore needed is a novel therapeutic strategy or unique material used to confer improved cellular neuron protection and significantly prevent, mitigate, or reverse toxic pathology arising from synaptic and neurological dysfunction before irreversible damage progresses. Desirably, such a treatment should prevent or avoid dyskinesia by including a means to remove sources of oxidation and free radical generation, to include a very localized and very targeted remediation of reactive oxygen species. It is believed the present invention provides the first broadly effective discovery of such a composition, having an intelligent biological and electrochemical design to confer multiple therapeutic and prophylactic functions that are highly targeted neural synaptic

structures, especially those in the substantia nigra of the brain. This novel composition will change our perspective on applications to boost resistance and generate recovery to the effects of Parkinson's and other motor neuron disease. The use of traditional carrier formulations will enable appropriate methods of administration for this novel composition.

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SUMMARY OF THE INVENTION

[0018] This invention is a molecular cluster of nanoparticles composed with fullerene levodopa gamma amino butyric acid or its metabolized form as fullerene dopamine gamma amino butyric acid. The use of dopamine (DOPA) as a starting material is interchangeable with, and
10 functionally equivalent to, the use of levodopa in the present invention and is hereby explicitly specified. The present invention is an inert mineral particle bonded with two types of neurotransmitters, where the gamma amino butyric acid (GABA) neurotransmitter functional group acts as a reducing agent with the tremendous advantage being that it behaves as an antioxidant to treat and proactively reduce the oxidative conditions leading to neurological disease
15 in neural synaptic structures associated with Parkinson's Disease, Lewy Body Disease, and Inflammatory Bowel Syndrome. Both GABA and the L-Dopa (or DOPA) functional groups or their metabolites confer neuroprotective properties. This composition is also useful to treat amyotrophic lateral sclerosis (ALS), and other neuron and motor neuron diseases or damages. The nanoparticle molecular structure possesses charge storage properties targeted to break plaque
20 forming regions using a salt-bridge disruption technology. The composition promotes free radical scavenging and targeted delivery to brain neurons in a synergistically enabled manner that is enabled by each functional group. The antioxidant properties of the functional groups are deliberately carried to the most oxidatively stressed region at neural structures, being the post synaptic bouton, while also providing a storage reservoir of reducing hydrogen protons on the C60
25 and the amine functionality of GABA to confer a localized chemical reducing condition.

[0019] In a key functional aspect, C60-GABA-L-Dopa and its metabolites to C60-GABA-DOPA provide an artificial pathway to supplement and accelerate the trafficking of cations for proton exchange to prevent or remove salt accumulation among oligomeric fibrils. This function acts to disassemble the oligomeric plaques formed by salt cations by extracting these cations, so
30 that they may not serve as salt bridges. This aspect of the invention depends on the use of the zwitterionic properties of the nanoparticle functional groups. C60 is normally considered anionic when it collects as many as six negative charges. The association of C60 with zwitterionic

functional groups has the additional properties of being an organic salt, in which both hydrogen bonding as well as aromatic pi to cationic pi bonding contributes to the stability of these structures and defines how this collective ensemble serves to traffic both protons and physiological cations such as potassium and sodium.

5 [0020] In another aspect, the C60-GABA-DOPA composition protects and enhances the membrane polarization of mitochondria by being able to penetrate them and protect them from oxidative stress. This allows protected mitochondria to significantly enable their normal ATPase function and undisrupted ability to generate reducing protons, where such hydrogen protons are then able to achieve reducing REDOX conditions at the neural post-synaptic terminal.

10 [0021] In a related aspect, the composition of this invention accrues and transports hydrogen protons to regions removed from the mitochondria where protons are required to exchange for physiological cations such as potassium, and sodium. This aspect can supplement endogenous substances fulfilling the same role.

[0022] In a related aspect, the free radical protective effect of the C60-GABA-DOPA on
15 mitochondria ensures the uninterrupted mitochondrial provision of chemically reductive protons. In a cascade effect, the produced protons act directly on dopamine molecules to enable them to maintain healthy individual alpha-synuclein fibrils in neurons. The functional individual alpha-synuclein fibrils then bond with the inner (cytosolic) leaflets of the presynaptic and post-synaptic membrane leaflets to stabilize the functional release and reacquisition of synaptic vesicles on
20 neurostimulation.

[0023] In a key aspect, the technological hurdle of supplying exogenously produced GABA neurotransmitter to the brain is provided by using a buckminsterfullerene (C60) carrier to enable crossing of the blood brain barrier and allow GABA's well known and outstanding medical benefits, including reducing blood pressure and enhancing long-term memory, to be directly
25 promoted to each brain region and all brain tissues.

[0024] In a related aspect, the transport of GABA into the brain by C60 allows it to be protected by the C60 functional group so that this form of GABA is unable to be easily broken down by neural enzymes, especially those released by astrocytes. This enhanced stability promotes the circulation of GABA with an extended lifetime or residence, in which it acts as both an
30 antioxidant and as a critically important neurotransmitter.

[0025] In another aspect, the provision of the GABA functional group on the C60-GABA-DOPA is to substitute for a lack of endogenously produced glutathione (GSH) antioxidant in

mitochondria. This replacement is neuroprotective to the mitochondria and acts to enable the ability of the mitochondria to return to a state of homeostasis, where it can now recycle the nanoparticles as modified exogenous neurotransmitters for release. The endogenously produced GABA may then bond with the lipids of the cellular membranes, including lipids at the outer
5 (intracellular) presynaptic membrane leaflets. The promoted presence of endogenously produced GABA acts in like manner to missing glutathione, as a reducing agent to prevent the accumulation of free radicals and oxidative damage to membrane lipids. This protection thereby prevents alpha-synuclein from otherwise forming toxic plaques by cross-linking reactions.

[0026] In another aspect, the presence of C60-GABA-DOPA is to penetrate those locations
10 in the neural structures already biochemically attractive to dopamine. The dopamine functionality of the introduced C60-GABA-DOPA is otherwise identical to and complementary with that of native or endogenous dopamine neurotransmitter. The advantages of this targeted delivery system are the highly localized delivery of GABA functionality as well as that of the fullerene groups to provide free radical quenching and powerful antioxidant functions to the lipid surfaces to those
15 oxidative locations where dopamine is required for proper neurotransmission, but in which GABA normally does not migrate, and in which C60 is never found except when externally provided.

[0027] The result of these combined functions is to allow time for the proper re-integration of dysfunctional or senescent neural cells by introducing an enhanced REDOX reversibility, and to directly inactivate reactive oxygen species (ROS) in mitochondria and at the surface membranes
20 of cellular organelles to re-establish functional cellular homeostasis.

[0028] In a related aspect, the function of the antioxidant fullerene GABA dopamine (C60-GABA-DOPA) is to correctively interact with alpha-synuclein oligomers arising from the otherwise pathological interaction with ordinary dopamine under dysregulated and oxidizing conditions. Thus, C60-GABA-DOPA functions as a dopamine mimetic, being functionally
25 identical to dopamine, and taking part in the same biochemical reactions as dopamine yet providing localized therapeutic reducing conditions critical to regulating neural cell function and restoring healthy neurotransmitter signaling at the synapse.

[0029] In yet another related aspect, the function of the antioxidant fullerene GABA dopamine (C60-GABA-DOPA) is to correctively detoxify and depolymerize oligomeric alpha-synuclein located in the vagus nerve and in and among the glia and neurons that control and regulate the digestive tract, thereby correcting ulcerative colitis, inflammatory bowel disease, and crone's disease types of pathologies.
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[0030] In yet another aspect, C60-GABA-DOPA disrupts sodium ion salt bridges between plaque fibrils to return individual strands of alpha-synuclein fibrils to their proper conformation and neurological function. Largely, it is the presence of the core fullerene molecule being tethered to a dopamine functional group that helps to disassemble detrimental salt bridges between proteins.

5 The high negative charge density acquisition of the fullerene group enables the abstraction and sequestering of sodium cations onto itself and away from the plaque proteins.

[0031] In another aspect, the C60-GABA-DOPA provides free radical quenching and antioxidant effects together with free radical recombination via the combined activity of both the GABA functional group and the fullerene C60 group, thereby ensuring a reducing rather than
10 oxidizing role in the presence of the metabolized dopamine functional group in this composition, to deter the formation of alpha-synuclein plaques, and to substantially avoid oxidized dopamine release of hydrogen peroxide to inflict damage on neural tissues.

[0032] In another aspect, the C60-GABA-DOPA composition is formulated to allow it to become sequestered into the pores of food grade Transcarpathian zeolite (clinoptilolite) for the
15 purpose of timed-release delivery of the orally administered composition to the digestive tract.

[0033] In another aspect, the C60-GABA-DOPA composition is administered in the form of a nano-aerosol for the purpose of immediate aspirated delivery to the lungs, thereby providing more direct access to the blood system for rapid release of the administered inhalant composition to the brain and bypassing the digestive system as well as any oxidative damage incurred by the
20 digestive tract fluids to the composition.

[0034] These and other advantages of the present invention will be further understood and appreciated by those skilled in the art by reference to the following written specification, claims, and appended drawings.

[0035] Some embodiments are described in detail with reference to the related drawings.
25 Additional embodiments, features, and/or advantages will become apparent from the ensuing description or may be learned by practicing the invention. In the illustrations, which are not drawn to scale, like numerals refer to like features throughout the description. The following description is not to be taken in a limiting sense but is made merely for describing the general principles of the invention.

30 **BRIEF DESCRIPTION OF THE DRAWINGS**

[0036] Preferred embodiments of the invention will now be described, by way of example, with reference to the accompanying drawings, in which:

- [0037] FIG. 1 is an illustration of some molecular structures of raw materials relevant to the teachings of the present invention.
- [0038] FIG. 2 is an illustration of molecular structures of the reactions of gamma amino butyric acid (GABA) with buckminsterfullerene (C60).
- 5 [0039] FIG. 3 is an illustration of the molecular structures of the reactions of levodopa (DOPA) with buckminsterfullerene (C60).
- [0040] FIG. 4 is an illustration of L-dopa, GABA, and C60 chemically reacting to synthesize C60-GABA-L-dopa having multiple aryl pi-pi bonds.
- [0041] FIG. 5 is an illustration of a metabolite of C60-GABA-L-dopa having some DOPA
10 functional groups, in which pi-carbonyl bonds, aromatic pi to aromatic-pi bonds, and hydrogen bonds create a molecular network structure.
- [0042] FIG. 6 is an illustration of alpha-synuclein plaques being intercalated with and disassembled by clusters of C60-GABA-L-dopa and / or metabolites thereof comprising C60-GABA-DOPA.
- 15 [0043] FIG. 7 is an illustration of clusters of C60-GABA-L-dopa and / or metabolites thereof comprising C60-GABA-DOPA providing protection and treatment at the neural synapse and at neural membranes.
- [0044] FIG. 8 is an illustration of the gut and the brain with alpha-synuclein or other prions reversibly migrating with neurotransmitters from the brain stem into somatic neural structures.
- 20 [0045] FIG. 9 is an illustration of a molecular structure for Transcarpathian zeolite (clinoptilolite) binder permeated or filled with C60-GABA-L-dopa.
- [0046] FIG. 10 is a flowchart representation of a synthesis of C60-GABA-L-dopa with a formulation for use as a nano-aerosol inhalant.
- [0047] FIG. 11 is a flowchart representation of a synthesis of C60-GABA-L-dopa with
25 formulations for oral administration.
- [0048] FIG. 12 is an illustration of personal administration of aspirated nano-aerosol C60-GABA-L-dopa.
- [0049] FIG. 13 is an illustration of an experimental FTIR data for levodopa (L-dopa).
- [0050] FIG. 14 is an illustration of an experimental FTIR data for buckminsterfullerene
30 levodopa (C60-L-dopa).
- [0051] FIG. 15 is an illustration of an experimental FTIR data for gamma amino butyric acid (GABA).

[0052] FIG. 16 is an illustration of an experimental FTIR data for buckminsterfullerene gamma amino butyric acid (C60-GABA).

[0053] FIG. 17 is an illustration of an experimental FTIR data for C60-GABA-L-dopa.

[0054] FIG. 18 is an illustration of an experimental negative mode mass spectrograph data
5 for C60-L-dopa.

[0055] FIG. 19 is an illustration experimental negative mode mass spectrograph data for C60-GABA.

[0056] FIG. 20 is an illustration of experimental negative mode mass spectrograph data for C60-GABA-L-dopa.

10 [0057] Some embodiments are described in detail with reference to the related drawings. Additional embodiments, features, and/or advantages will become apparent from the ensuing description or may be learned by practicing the invention. In the illustrations, which are not drawn to scale, like numerals refer to like features throughout the description. The following description is not to be taken in a limiting sense but is made merely for describing the general principles of
15 the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0058] The following detailed description, taken in conjunction with the accompanying drawings, is merely exemplary in nature and is not intended to limit the described embodiments or the application and uses of the described embodiments. Any implementation described herein
20 as “exemplary” or “illustrative” is not necessarily to be construed as preferred or advantageous over other implementations.

[0059] Furthermore, there is no intention to be bound by any expressed or implied theory presented in the preceding technical field, background, brief summary or the following detailed description. It is also understood that the specific devices, systems, methods, and processes
25 illustrated in the attached drawings, and described in the following specification, are simply exemplary embodiments of the inventive concepts defined in the appended claims that there may be variations to the drawings, steps, methods, or processes, depicted therein without departing from the spirit of the invention. All these variations are within the scope of the present invention. Hence, specific structural and functional details disclosed in relation to the exemplary
30 embodiments described herein are not to be interpreted as limiting, but merely as a representative basis for teaching one skilled in the art to variously employ the present embodiments in virtually

any appropriate form, and it will be apparent to those skilled in the art that the present invention may be practiced without these specific details.

[0060] Various terms used in the following detailed description are provided and included for giving a perspective understanding of the function, operation, and use of the present invention, and such terms are not intended to limit the embodiments, scope, claims, or use of the present invention.

[0061] FIG. 1 illustrates molecular structures 10 used or metabolized in the composition of the present invention. Dopamine (DOPA) 11 has the chemical formula $C_8H_{11}NO_2$ and is also known as the endogenous neurotransmitter 3,4-dihydroxyphenethylamine. Levodopa (L-dopa) 12 is an amino acid of chemical formula $C_9H_{11}NO_4$ that is commercially available as a synthetic food supplement and is readily metabolized by decarboxylation to form the neurotransmitter dopamine (DOPA) 11 as well as other neurotransmitters. It is generally understood and recognized that L-dopa 12 is a chief chemical precursor to DOPA 11 and may be used in neuroprotective treatments for Parkinson's Disease, inflammatory bowel disease, and other neurological disorders. The molecular structure 14 is gamma aminobutyric acid (GABA) and has the chemical formula $C_4H_9NO_2$. GABA is a major inhibitory neurotransmitter synthesized and delivered by GABAergic neurons but has seen limited usefulness because of very poor blood brain barrier diffusion from outside the brain and intensive breakdown by astrocyte GABA transaminase from inside the brain. Buckminsterfullerene 16 is a single molecule comprised of 60 carbon atoms arranged as a sphere and has the chemical formula of C_{60} . Substances 11, 12, 14, 16 may be used to help create, process, or deliver parts of the composition of C60-GABA-L-dopa.

[0062] FIG. 2 illustrates molecular structures of two chemical reaction pathways 20 of gamma amino butyric acid (GABA) 23 with buckminsterfullerene (C_{60}) 21. At elevated temperatures being above $120^\circ C$, the direction of the reaction pathway follows the white arrow to produce at least one covalent bond 28 between the at least one GABA nitrogen functional group and the C_{60} functional group to form a covalently bonded GABA- C_{60} 24. This high temperature reaction pathway is undesirable because it removes the neuroprotective effect of the amine functional group. At room temperature being below at most $40^\circ C$, the direction of the reaction pathway under high pressure shear conditions, substantially follows the solid black arrow to produce at least one aromatic pi to carbonyl bond 27 between the at least one GABA carbonyl functional group and the C_{60} functional group, forming the configurational isomer of GABA- C_{60} ,

26 having the preferred geometry in which the amine nitrogen of GABA is free to act as a reducing agent against oxidants in a neuroprotective manner.

[0063] FIG. 3 illustrates molecular structures of two chemical reaction pathways 30 of L-levodopa (L-dopa) 32 with buckminsterfullerene (C60) 31. At elevated temperatures being above 5 120 °C, it is well known that the direction of the reaction pathway follows the white arrow to produce at least one covalent bond 34 between the at least one L-dopa nitrogen functional group, or at the carboxylic acid functional group, to react with a carbon atom of the C60; this type of reaction is to be avoided at the C60 functional group, because these two covalently bonded configurational isomers of L-dopa do not ensure the preservation of a labile and neurologically 10 available amine adduct in accordance with the molecular design specified herein. The pi-carbonyl bonded L-dopa with C60 33 is capable of being achieved under shear mixing conditions and at room temperature or below at most about 40 °C. This low temperature and high shear pressure reaction is the direction of the reaction pathway that follows the solid black arrow to produce aromatic pi to carbonyl bond 36 and / or an aromatic-pi to aromatic-pi bond between the at least 15 one GABA functional group and the C60 functional group, being GABA-C60 37 having the preferred adduct geometry in which the amine nitrogen of L-dopa is free to act as a reducing agent against oxidants in a neuroprotective manner.

[0064] FIG. 4 illustrates levodopa (L-dopa) 43 and GABA pi-carbonyl 42 reactions 40 with buckminsterfullerene (C60) 41, to generate the products shown in the direction of the large black 20 arrow. In humans, metabolic conversion of L-dopa 43 to dopamine occurs by loss of the carboxyl (-COOH) functional groups 49a, 49b within the cells of the central nervous system as well as in the motor neurons of the peripheral nervous system. Administering L-dopa 43 alone can lead to excessive undesirable neural signaling and may also cause many of the adverse side effects associated with Dyskinesia under conditions of oxidative stress. The multiplicity of x molecules 25 of GABA at 44 is denoted by the subscript letter x after the molecular structure within the bracketed region. The multiplicity of y molecules of L-dopa at 47 is denoted by the subscript letter y after the molecular structure within the bracketed region having chemical formula $C_9H_{11}NO_4$. Nominally, x is 9 and y is 6, where it is understood that the 6 moles of L-dopa are metabolized to the neurotransmitter dopamine (DOPA) when in the form of the metabolized functional group as 30 C60-GABA-L-dopa becomes C60-GABA-DOPA and enters the neural tissues. The aromatic pi-pi bond with the aromatic regions of C60 48 represented by dashed line 46 has more molecular structural strength than hydrogen bonds but is weaker than a covalent bond. Aromatic pi to

carbonyl bond represented by dashed line 45 has more molecular structural strength than hydrogen bonds but is weaker than a covalent bond. It is to be understood that L-dopa 43 or the multiplicity of y molecules of L-dopa functional groups 47 will metabolize via decarboxylation to form the neurotransmitter DOPA of chemical formula $C_8H_{11}NO_2$ as the new adducts 47 as an intended form of the active ingredient of the present composition.

[0065] FIG. 5 illustrates the molecular structures 500 leading to formation of a networked C60-GABA-L-Dopa with C60-GABA-DOPA after partial decarboxylative metabolism of some of the L-dopa functional groups. A multiplicity of hydrogen bonds is represented by dotted lines, such as 560, 580, 581, 582 in these structures. A multiplicity of pi-bonds is illustrated in these molecular structures as dashed lines extending outward from the C60 groups 510, 540 by representative 520, 550. It is to be noted that functional groups of dopamine (DOPA) 560, 590 have chemical formula $C_8H_{11}NO_2$ and are also known as the endogenous neurotransmitter 3,4-dihydroxyphenethylamine. DOPA is by means of enzymatic metabolism, partly replacing the residual functional groups of levodopa (L-dopa) 530, 550, an amino acid of chemical formula $C_9H_{11}NO_4$, which is a substance that is commercially available as a synthetic food supplement when present in pure form. Each levodopa (L-dopa) 530, 550 functional group on C60, 510 is readily metabolized by decarboxylation to form the functional group dopamine (DOPA). The GABA functional group 570 is shown, connected by a hydrogen bond 582 to L-dopa 590, which represents the type of association found or produced in a natural synapse and forms on the reversible release of its former pi-carbonyl bond to C60 illustrated in FIG. 4.

[0066] Pi-carbonyl bonded functional groups of GABA 571, 573 represented by R1, R2 have formula $C_4H_9NO_2$, where each of these is a zwitterion at physiological and neutral pH, meaning that the proton from the carboxylic acid group can leave and become associated by hydrogen bonding to the amine nitrogen functional group at the opposing distal end of this structure as is represented by GABA 570. GABA 570 reacts with a multiplicity of substituted C60 510, 540 having at least one pi-bonded dopamine functional group 560, 590 or at least one levodopa functional group 530, 550 to form the derivative C60-GABA-L-dopa provided with a multiplicity of GABA functional groups. As both GABA and L-dopa are zwitterions, and as C60 is normally considered anionic when it collects as many as six negative charges, the association of C60 with these zwitterions has the properties of being an organic salt, in which both hydrogen bonding as well as aromatic pi bonding contribute to the stability of these structures. Composition variations may be tuned by the number but not the type of functional groups, depending on

penetrating and trafficking function, and may be from at least one GABA and at least one L-dopa to about 9 GABA and about 6 L-Dopa, in which C60 bonded with 2 DOPA and 3 GABA functional groups presents adequate and sufficient medical improvement in human Parkinson's disease. The dual neurotransmitter functionality of GABA and DOPA adduct with C60 is the novel
5 neurotransmitter structure of C60-GABA-DOPA. It is to be understood that the fully decarboxylated metabolite on reaction completion in which C60-GABA-DOPA resides in the brain is the final metabolized form of this composition which performs therapeutic functions, according to the teachings of the present invention.

[0067] FIG. 6 illustrates the role of metabolized C60-GABA-DOPA to disassemble the
10 toxic oligomeric plaque of alpha-synuclein 60. A substantially one-dimensional fibril of alpha-synuclein 61 tends to form lengthwise abutting bonds with a multiplicity of other alpha-synuclein fibrils termed more generally a plaque 62. The type of bonding along adjacent fibril lengths can include van-der-Waals induced charges, however salt cations such as sodium 64 may also intercalate or squeeze between these fibrils to create tangles that increase in size with time;
15 oxidative species may additionally interpose cross-links and protein functional groups into random locations of the alpha-synuclein fibrils to include aldehydes or carboxylic acids under oxidative conditions. Free radical additions may also form bonds between fibrils when free radicals are present.

[0068] The introduction of clusters containing C60-GABA-DOPA 62, 63 into and among
20 alpha-synuclein plaques 62 allows the quenching of free radicals and provides anti-oxidant functionality. Clusters containing C60-GABA-DOPA 62, 63 also store and then release hydrogen protons 66 carried at the amine nitrogen of dopamine or GABA functional groups, wherein up to about five additional hydrogen protons 66 may be carried by the fullerene C60 functional group. Fullerenes are also known for their ability to store as many as six negative charges, whereby the
25 high negative charge concentration in the clusters of C60-GABA-DOPA 62, 63 can extract sodium cations 64 from plaque 62, thereby freely releasing individual alpha-synuclein fibrils 61 from the collective plaque tangle 62. The combination of free-radical quenching, anti-oxidant function, cationic extraction, and free proton release enables the proper function of the dopamine neurotransmitter. The targeting of reductive GABA functional groups from C60-GABA-DOPA to
30 those oxidative locations at the post-synaptic terminal where GABA is needed to counteract the oxidative stress, is accomplished by the chemical affinity of the dopamine ligands within the C60-GABA-DOPA clusters 62, 63. The provision of C60 fullerene as a multifunctional center for these

chemistries, in addition to the role of C60 as a hydrogen storage functional group, help to create local reducing conditions amenable to prophylactic neural protection from oxidative stress induced degradation prevention.

[0069] In the cellular lysosomes and other cellular vesicles, hydrogen protons 66 are
5 exchanged with sodium 64, potassium 65, and other cations by various endogenous early
endosome sodium-hydrogen exchangers such as NHE6. Medical evidence of genetic defects in the
proteins used to traffic these cations are associated with the oligomeric agglomeration of plaques
such as found in Alzheimer's Disease, some forms of autism, and Christianson syndrome. Alpha
synuclein is yet another oligomer that has confirmed salt bridges. Alpha-synuclein needs to be
10 present as individual fibrils to transport cations to biological membranes; a multiplicity of salt
bridge hydrogen bonds are represented by the dotted lines 67 to bind the oligomer fibrils together
so that they may no longer perform their cation shuttling function. C60-GABA-DOPA functions
to artificially accelerate the trafficking of cations for proton exchange using a prosthetic pathway
that prevents salt accumulation among the oligomeric fibrils, disassembles the oligomeric plaques
15 formed by salt cations, and extracts the salt cations 64, 65 from alpha synuclein so that cations
may not serve as salt bridges. The clusters of C60-GABA-DOPA 62, 63 constitute a prosthetic
dual neurotransmitter having properties of both GABA and DOPA to enable this neural disease
treatment according to the teachings of the present invention.

[0070] FIG. 7 illustrates the role of alpha-synuclein at a synapse and at some of the
20 organelles of a neuron 700. It is well understood that alpha-synuclein binds to and regulates the
transfer of calcium ions 766, 767, especially those that are pooled and clustered within the synaptic
vesicles released from the presynaptic terminal 764 during neurotransmitter release at the synaptic
junction 760 between two neurons 710, 750. Alpha synuclein also influences the regulation of the
vesicle trafficking from the endoplasmic reticulum 742 to the cell membrane at dendrites 744, and
25 in vesicle adhesion to the Golgi complex 735 and neural cell nucleus 730. Alpha-synuclein
localizes at the mitochondrial membranes 737, where it mitigates the effects of oxidative stress.
These functions are enabled by the free radical, antioxidant, hydrogen proton storage, and cation
trafficking composition of the C60-GABA-DOPA clusters 746, 768 that complement endogenous
cation porter molecules in the manner of neurotransmitters, and thereby act to maintain the non-
30 plaque independent fibril form of alpha-synuclein, as well as to establish cellular homeostasis
among neurons.

[0071] Filopodia 720 are slender cytoplasmic neural projections that extend beyond a first neuron 710 and may have at least one synaptic junction 760 with a second neuron illustrated as a partial section of another filopodium extension 750. At least one metabolized C60-GABA-DOPA cluster 768 has been reduced in size to about less than 35 nanometers as part of the metabolic process, which enables it to enter the synaptic cleft between pre-synaptic vesicle 764 and post synaptic terminal 762. Cluster 768 provides multifunctional roles to stabilize the membrane lipid interaction at the synaptic junction 760 where neurotransmitter 766 accumulates within the presynaptic terminal as neural bouton 764 for release into the synaptic gap 767 to be received by neural receptors at the proximal neuron providing the post synaptic terminal 762. Vesicles such as 764 may detach and travel with neurotransmitter 767 while carrying charged cations such as Na⁺ and Ca²⁺, wherein independent alpha-synuclein fibrils are critical to maintain the multiplicity of cations as adducts. The redox chemistry homeostasis provided by C60-GABA-DOPA clusters 746, 768 destabilizes plaques by the prevention of the free-radical and oxidative kinetics of alpha-synuclein aggregation, and by extracting cations from between alpha-synuclein fibrils, thereby halting or reversing the formation of oligomeric proteins aggregates and their associated toxicity, according to the teachings of the present invention.

[0072] FIG. 8 illustrates the gut and the brain with alpha-synuclein or other toxic oligomeric plaques that reversibly migrate with neurotransmitters from the brain stem into somatic neural structures. Migrating toxic oligomeric plaques 870, 840 affecting cognitive processes, autonomic control, hearing, vision, the digestive tract 890, and deliberate conscious muscular control are coordinated by and at the human brain 810, reversibly diffuse with neurotransmitters along the central nervous system (CNS) along and through the brain stem 850 to propagate along neurons such as the vagus nerve 860, or other nerves such as the spinal cord (not shown). The human brain 810 is semi-permeably separated from the vasculature fluids by a barrier well known as the blood-brain barrier or BBB; a functionally similar barrier exists between the bacteria inside the digestive tract 890 and the neurons and glia 830 which control the percolation of media through digestive system, where a cross section of one part of this barrier is shown in the enlarged inset view 820. The presence of toxic alpha-synuclein plaques act to destroy some cells and create large openings among a multiplicity of semi-permeable cells of the gut barrier lining 880, thereby allowing some of the gut bacteria to penetrate the gut barrier lining 880 through gaps or holes in the tight binding junction between cells of the gut barrier lining 880 in the direction of the upward facing black arrow at inset view 820. In some cases of digestive disorder, blood may then flow

from the region of the vasculature near to or abutting the glia 830 to the bacteria and stool reservoir within digestive tract 890. The erosion or death of some of the gut lining cells 880 is caused by the interaction with alpha-synuclein oligomers 840, under medical conditions that may be associated with dysbiosis, where some of these conditions are inflammatory bowel disease leading in some cases to ulcerative colitis, Parkinson's disease, and Lewy Body Disease. The loss of dopamine and GABA (gamma amino butyric acid) neurotransmitters as well as alpha-synuclein from the region of the glia 830 through eroded places among the tight binding junctions of proximal and abutting gut lining cells 880 is symptomatic of a 'leaky gut', and may contribute to the formation and propagation of oligomeric alpha-synuclein plaques 840, where such plaques can in some cases migrate along and among neurons such as the vagus nerve 860 to propagate and return such oligomeric plaques to the brain 810 to accrue cognitive dysfunctions that cause pathologies and a state of disease.

[0073] Molecules 888 of the present invention may be injected into the blood using a standard physiological saline solution of from about 0.1 mg/Kg to about 5 mg/Kg, consumed as an oral dosage, or inhaled as a nano-aerosol through the lungs to enter the blood stream directly. The presence of negative electrostatic pi-anionic charges among molecules 888 serves to neutralize the electrostatic cation-pi charges among a multiplicity of toxic oligomeric plaques and prions or prion-like particles 840 to disassemble these agglomerates, whereby the healing process among the affected cells can immediately begin to close the gap among the barrier cells 880 at a multiplicity of gaps or holes as indicated by the large upward facing black arrow to once again establish the healthy condition of contiguous tight binding barrier junctions, in accordance with the teachings of the present invention.

[0074] FIG. 9 illustrates zeolite impregnated with a C60-GABA-L-dopa 90. Transcarpathian zeolite (clinoptilolite) 91 is a type of mineral provided with a highly negative charged network structure achieving a system of reproducible and well-defined pores and channels. Clinoptilolite zeolite 91 is well known to adsorb oppositely charged nitrogen containing compounds including protonated ammonia and protonated amino acids which serve as positive counter-ion and hydrogen bonding adducts with the composition of C60-GABA-L-dopa molecules agglomerated in the form of clusters 92, 93, 94, 95, 96, and 97 having sizes sufficiently small to fit within the mineral scaffold, where the channels therein can typically range from greater than 100 nanometers to less than about 5 microns in size. It is also known that at pH greater than 7, as well as under saline or physiological ionic salt conditions, clinoptilolite zeolite displaces and

expresses the positively charged nitrogen compounds and counterions stored within the channels of zeolite 91. The salt and pH moderated regenerant property of zeolite 91 towards reversible expression and release of positively charged nitrogen compounds has led to the widespread economic commercial adoption of clinoptilolite Transcarpathian zeolite 91 as a dietary supplement. It is therefore specified to utilize this ion-exchange property of zeolite 91 as one exemplary method to perform a timed-release function of the C60-GABA-L-dopa composition of the present invention as a practical and cost-effective method of delivering and administering this composition, according to the teachings of the present invention.

[0075] FIG. 10 is a flowchart representation of a synthesis and nano-aerosol formulation of C60-GABA-L-dopa 100. In step 101 at least about one and nominally 3 molar equivalents of pure GABA are combined with one molar equivalent of vacuum purified buckminsterfullerene (C60) and at least about one and nominally 2 molar equivalents of pure levodopa (L-dopa). In step 102, the dry powder mixture is reactive shear-milled at greater than 1000 per second shear rate at a processing temperature below 40 °C to minimize the covalent bonding of amine groups from the GABA or the L-dopa onto the C60, while maximizing the pi-carbonyl and pi-aromatic bonding with C60. One way that low temperature processing can be accelerated at higher shear rates for less time, is to provide an oxygen free processing atmosphere. In step 103 the sheared C60-GABA-L-dopa product is added to polypropylene glycol (PPG) solvent in a 1:10 mass ratio of dry powder to solvent for liquid shear at about 1000 per second shear rate to full product dissolution. In step 104, the desired concentration of C60-GABA-L-dopa is created by dissolving a volumetric amount of the C60-GABA-L-dopa solution into a solvent mixture of glycerol with polypropylene glycol to achieve the desired final concentration of between about 20 ppm and 2000 ppm to obtain a suitable vaporized inhalant or a dosage for nano-aerosol inhalant delivery. This final dilution solvent mixture comprises about 70% glycerol and 30% polypropylene glycol by volume. All solvated components for dispensing are to be kept free of moisture in a quality-controlled process. In step 105, a metered amount of the nano aerosol is generated by a commercially available electronic dispensing device, such as by heating the formulated fluid at from about 255 °C up to about 300 °C, but no greater than about 300 °C to avoid oxidation or breakdown of the nano-aerosol, and to maintain temperatures suitable for client aspiration, according to the teachings of the present invention.

[0076] FIG 11 is a flowchart representation of a synthesis of C60-GABA-L-dopa and a formulation for Oral Administration 110. In step 111 at least about one and nominally 3 molar

equivalents of pure GABA are combined with one molar equivalent of vacuum purified buckminsterfullerene (C60) and at least about one and nominally 2 molar equivalents of pure levodopa (L-dopa). In step 112 the dry powder mixture is shear milled at greater than 1000 per second shear rate while the processing temperature is maintained below 40 °C to minimize the covalent bonding of amine groups from the GABA or the L-dopa onto the C60, while maximizing the pi-carbonyl and pi-aromatic bonding with C60. One way that low temperature processing can be accelerated at higher shear rates for less time, is to provide an oxygen free processing atmosphere. In a first alternative step 114, a desired quantity of hydrogen bonded C60-GABA-L-dopa powder product obtained from step 113 is dissolved into aqueous 0.1% to 0.3% hyaluronic acid, then desired colors, flavors, and preservatives such as potassium sorbate or sodium benzoate are added for oral administration or beverage servings. In a second alternative step 115, the C60-GABA-L-dopa powder product is combined with one or more pharmaceutically acceptable carriers like suitable USP food grade binders as delivery materials in any combination. These carriers and delivery materials are generally known as excipients and fillers, of which non-limiting examples include commercially available calcium carbonate, zeolite, methyl cellulose, and gel peptides for placement into a compressed tablet or a gel capsule as desired for oral administration, according to the teachings of the present invention.

[0077] FIG. 12 illustrates a personal administration method 120 for an aspirated nano-aerosol delivery system containing an C60-GABA-L-dopa composition. The nano-aerosol generating device filled with C60-GABA-L-dopa dispensing solution 128 is provided for dispersing the inhalant gas wherein the nano-particles are and nebulized. The dispensing method of commercially available device 128 may also be more commonly known as a nebulizer, or an electronic vaporizing device, or an electronic cigarette, or the functional part of a hookah to be shared among several users. In all cases these systems serve to carry the C60-GABA-L-dopa in a carrier fluid dispenser 128, move that composition in nebulized form along with an aerosolized solvent, and transfer this composition in substantially gaseous dispersion to the nose, mouth, trachea, and airways of a patient or user 127. One intended use of the C60-GABA-L-dopa composition is to treat, delay or arrest the incidence of Parkinson's disease (PD), Alzheimer's disease (AD), and other cognitive disorders wherein the nano-aerosol can expedite targeted delivery to the brain by avoiding a passage through the digestive system.

[0078] Some of the nano-aerosolized composition is exhaled and shown as particulate clusters 121, 122, 123 within exhaled smokey puffs 124 and 125 emitted on exhalation as indicated

by the direction of thin line arrows radiating away from the nose of the subject 127. Delivery of the C60-GABA-L-dopa nano-aerosol composition from dispenser 128 provides antioxidant properties to the mucus airway tissues wherein destruction of free radicals and oxidants associated with motor neuron disease and Parkinson's disease are part of the treatment and alpha-synuclein plaque mitigation is provided using this method. Systems that may be used for the method of dispersion of the C60-GABA-L-dopa represented by dispenser 128, include, without limitation, any of the electronic cigarette devices produced internationally and listed in Appendix 4.1, "Major E-cigarette Manufacturers" of the "2016 Surgeon General's Report: E-Cigarette Use Among Youth and Young Adults" published by the Center for Disease Control and Prevention (CDC), Office of Smoking and Health (OSH) freely available at the CDC.GOV website, and / or any combination of piezoelectric, resistively heated, or inductively heated vaporized fluid delivery methods that can be utilized to deliver the composition of the present invention, especially when approved as a medical drug delivery device. Each embodied variation of such methods without limit are intended to aspirate aerosols as the method of therapeutic substance delivery of the composition of the present invention directed into the nasal cavities, mouth, tracheal breathing orifice, or intubated trachea of a patient. The supply direction of nebulized feed of C60-GABA-L-dopa on inhalation and exhalation are delivered into the airways and lungs of the intended patient by the flow of supplied air as indicated by the direction of upward and downward facing large white arrows 126, when used according to the teachings of the present invention.

[0079] FIG. 13 illustrates experimental FTIR data for levodopa. All the Fourier transform infra-red (FTIR) spectrographs hereinafter were measured by transmittance using the potassium bromide (KBr) compressed flow solid pellet compact preparation method. The material used for analysis was obtained by the method of mixing, crushing, and consolidating under 7 metric tons of pressure, about 0.001 grams of the analyte substance with 1 gram of a diluent solid KBr that is substantially transparent to infrared light, and which flows under pressure to form a translucent pellet of about 0.4 mm thickness. Spectral background subtraction in air using a control pellet of the same mass and thickness having pure KBr was used to obtain a baseline instrument infrared spectral response. This method is generally referred to as the 'KBr pellet' sample preparation method, and it is used hereinafter throughout for each FTIR experimental data collection and spectral analysis. The Fourier transform infrared spectrophotometer used herein to obtain FTIR spectra throughout, is a model RF6000 FTIR instrument manufactured by Shimadzu of Japan.

Each FTIR data graph hereinafter is provided with a numeric scale ranging from 400 to 4000 to represent reciprocal centimeters or (cm-1) in wavenumbers.

[0080] The numeric scale ranging from 10 to 90 represents percentage transmittance and has units of percentage (%). The FTIR absorbance peak at 3359 cm-1 is attributed to the amine nitrogen-hydrogen vibration (N-H). At 3200 cm-1 appears an oxygen-hydrogen (O-H) stretching vibration, and at 3046 cm-1 is an aromatic hydrogen stretching vibration. The primary amine functional group is indicated by the two (N-H) bending absorbance vibration bands at 1653 cm-1 and at 1567 cm-1. The peaks between 1064 cm-1 and 1200 cm-1 are due to (C-N) stretching vibrations. The sharp and intense peak at 817 cm-1 indicates the N-H bending vibration. There is evidence of a band at about 1500 cm-1 that can be attributed to the C=C bond in the benzene ring structure. Comparison of the illustrated experimental FTIR data for levodopa 1200 indicates similarity to the FTIR absorbances reported for levodopa that are available from the scientific literature, and may be used for confirmation of the raw material composition according to the teachings of the present invention.

[0081] FIG. 14 illustrates experimental FTIR data for fullerene C60 reacted with levodopa, being C60-L-dopa. The numeric scale ranging from 30 to 100 represents percentage transmittance and has units of %. The characteristic strong and sharp buckminsterfullerene (C60) aromatic carbon-carbon stretching band is present at 526 cm-1. The FTIR absorbance peak at 3373 cm-1 is attributed to the amine nitrogen-hydrogen vibration (N-H). At 3192 cm-1 appears an oxygen-hydrogen (OH) stretching vibration, and at 3062 cm-1 is an aromatic hydrogen stretching vibration. The two bands arising from the primary amine functional group are indicated by the (N-H) bending absorbance vibrations and remain unchanged at 1653 cm-1 and at 1567 cm-1, confirming that there was no chemical reaction to alter the amine functional group. The peaks between 1064 cm-1 and 1200 cm-1 are due to (C-N) stretching vibrations. The sharp and intense peak at 821 cm-1 indicates the N-H bending vibration. The observed reduction of absorbance intensity in the region of 1450 cm-1 to 1500 cm-1 can be attributed to the attenuation of the C=C bond vibrations in the levodopa benzene ring that has become sterically constrained by aromatic-pi bonding with the C60 functional group, wherein the spatially confined geometry impacts the bond mobility in bending and stretching modes associated with the formation of the aromatic pi to aromatic pi bonds.

[0082] FIG. 15 illustrates experimental FTIR data for GABA raw material that was used to synthesize the compositions of the present invention. The numeric scale ranging from 0 to 100

represents percentage transmittance and has units of %. The FTIR absorbance peak at 3416 cm⁻¹ is attributed to the amine nitrogen-hydrogen vibration (N-H). The protonated amine group (NH₃⁺) results in the observation of broad multiple peaks of the gamma aminobutyric acid spectrum in the 3300 cm⁻¹ to the 2600 cm⁻¹ range. It is notable the band at around 2125 cm⁻¹ has been associated with an amine hydrogen (N-H) stretching of zwitterionic salts. The absorption at 1563 cm⁻¹ indicates the presence of carboxyl functional group (C=O) symmetric stretching vibration that is characteristic of the GABA molecule. The strong and sharp peak observed at 1396 cm⁻¹ is attributed to a deprotonated oxygen as part of the carboxylic acid (COO⁻) in an asymmetric vibration mode of this functional group. This confirms the zwitterionic state of GABA by FTIR.

The overall infrared absorbance spectral features are consistent with and indicate chemical similarity to GABA as may be found in published public FTIR spectra for this raw material, according to the teachings of the present invention.

[0083] FIG. 16 illustrates experimental FTIR data for buckminsterfullerene gamma aminobutyric acid C60-GABA. The numeric scale ranging from 0 to 100 represents percentage transmittance and has units of %. It is notable the band at around 2125 cm⁻¹ has been associated with an amine hydrogen (N-H) stretching of zwitterionic salts. A negatively charged ion or anion in this material is buckminsterfullerene (C60), which is known to accrue a charge of as many as six electrons. Also quite notable is the appearance of an enhanced absorption for protonated amine hydrogen (N-H) peak at 2946 cm⁻¹ in which this result supports the formation of a counter-ionic species that is reminiscent of the vibrational properties of a salt. It is also a characteristic of C60 to have a strong affinity to store protons as counter-charges. Therefore, the strengthening of the band at 2496 cm⁻¹ compared to the same region for GABA in FIG. 14 is attributed to the anionic C60 salt of GABA contribution. The absorption at 1563 cm⁻¹ indicates the presence of carboxyl functional group (C=O) symmetric stretching vibration has not changed this overall characteristic of the GABA molecule. The strong and sharp peak observed at 1396 cm⁻¹ is attributed to a deprotonated oxygen group as part of the carboxylic acid (COO⁻) group and remains as an asymmetric vibration mode of this functional group. These features are consistent with a zwitterionic C60 organic salt of GABA by FTIR as a distinguishable material having clearly recognizable chemical features as a chemical intermediate that is formed in the process of the chemical synthesis of the composition of the present invention.

[0084] FIG. 17 illustrates experimental FTIR data for C60-GABA-L-dopa. The numeric scale ranging from 0 to 100 represents percentage transmittance and has units of %. The absorption

peak at 526 cm⁻¹ is characteristic for C60 fullerene carbon-carbon (C=C) bonds. The protonated amine functional group (NH₃⁺) at 3424 cm⁻¹ attributed to the zwitterionic salt formation with an anionic C60 stabilized counterion to the hydrogen bonded protons dominate this entire region of the infrared spectrum so strongly that it overrides the multiple shoulders of peaks at 3252 cm⁻¹, 3031 cm⁻¹, and 2919 cm⁻¹ attributed to a combination of levodopa and GABA amine functional contributions. The intense sharp absorbance at 1653 cm⁻¹ indicates the presence of a carboxyl functional group (C=O) symmetric stretching vibration that is more characteristic of the levodopa molecular functional group, while the retention of the 1564 cm⁻¹ indicates the contribution of a different carboxyl (C=O) molecular functional group attributed to the presence of GABA. The peak observed at 1406 cm⁻¹ is attributed to a deprotonated oxygen as part of the carboxylic acid (COO⁻) in an asymmetric vibration mode of this functional group, but it is shifted to indicate a substantially different chemical environment exists than in that of any of the other FTIR characterizations provided herein. The disappearance or significant attenuation of the carbon-carbon (C=C) ring vibrations at about 1458 cm⁻¹ is attributed to strong geometric or steric hindrance associated with significant aromatic pi to aromatic pi bonding between C60 and the levodopa benzene ring structure. This confirms the unique chemical signatures of combined zwitterionic states of levodopa (L-dopa) and GABA interacting as functional groups in combination with an organic salt complex formed with C60 by FTIR as a distinguishable material having clearly recognizable chemical signatures that are characteristic of the composition of the present invention.

[0085] FIG. 18 illustrates experimental negative mode MALDI-TOF mass spectrograph data for C60-L-dopa material 1800. The ratio of mass to charge (m/z) is used to determine the molecular ion fragments to help determine the pieces of the original molecule in this assay. The mass peak at 723 m/z corresponds to the molecular ion fragment of fullerene C60 of mass 720 having three adducted hydrogen atoms. The very broad mass peaks at 1370, 2042, and 2641 are attributed to indicate predominantly dimeric and some trimeric C60 chains appended to each other and to interstitial levodopa by pi-pi bonding. The rider peaks on the broader peaks indicate the loss of small ion fragments such as those having a mass of 17 from (-OH) hydroxyl groups. The overall experimental test results characterize the molecular ion breakdown products of C60-L-dopa, where C60-L-dopa may be used to further synthesize the composition of the present invention.

[0086] FIG. 19 illustrates experimental negative mode MALDI-TOF mass spectrograph data for C60-GABA material 1900. This test sample resulted from reacting an equivalent molar

quantity of GABA to the molar equivalent of pristine buckminsterfullerene C60. The mass peak at 721 m/z corresponds to the molecular ion fragment of fullerene C60 of mass 720 having one adducted hydrogen atom. The primary molecular ion was subsequently verified using a pristine pure reference material of C60 tested immediately after this test, under both negative mode and positive mode test conditions (results are not shown here). The observed molecular fragment at 328 is attributed to a trimeric GABA zwitterion with the inclusion of a water molecule and a proton. The observed peak shoulder having a molecular fragment at 866 is characteristic for a fullerene C60 obtaining a residual spallation fragment from GABA that was incompletely removed. The cluster of peaks with a maximum at 1420 is attributed to C60-GABA, wherein one molecular mass of GABA is bonded to one molecular mass of buckminsterfullerene. The presence of peak clusters at 2138 and 2828 are evidence of trimeric and tetrameric network structures of C60 provided with bridging GABA functional groups. The overall result serves as a fingerprint to characterize the C60-GABA component that may be used as an intermediate to further synthesize the composition of the present invention.

[0087] FIG. 20 illustrates experimental negative mode MALDI-TOF mass spectrograph data for negative mode mass spectrograph data for C60-GABA-L-dopa 2000. The mass peak at 721 m/z corresponds to the molecular ion fragment of fullerene C60 of mass 720 having one adducted hydrogen atom. The GABA ion spallation fragment of 866 in FIG. 19 is also seen illustrated here but it is present as a trace quantity and may be difficult to distinguish unless it is a subjected to scrutiny for a search and confirmation in the test data. Likewise, is the observed molecular fragment at 329 attributed to a trimeric GABA zwitterion with the inclusion of a water molecule and two protons, but this result is also attenuated and may be difficult to distinguish unless it is a subjected to scrutiny for a search and confirmation in the test data.

[0088] The first broad cluster of peaks present at 1445 m/z is consistent with 3 GABA functional groups and two L-dopa functional groups on one C60 group. It is to be understood that adding more functional groups at the time of sample synthesis will change the average mass of the clustered peak grouping but will not fundamentally change the design of the composition or the identity of the pendant groups. The lack of significant dimeric, trimeric, or tetrameric molecular ion fragments indicates that for this number and composition of pendant groups, there is no significant molecular network structure for this sample of C60-GABA-L-dopa. The overall illustrated mass spectral fingerprint of molecular ion fragments 1900 sufficiently characterizes one embodiment of the C60-GABA-L-dopa molecular system according to the teachings and composition of the present invention.

[0089] As variations, combinations and modifications may be made in the construction and methods herein described and illustrated without departing from the scope of the invention, it is intended that all matter contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative rather than limiting. Thus, the breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments but defined in accordance with the foregoing claims appended hereto and their equivalents.

CLAIMS

What is claimed is:

- 5 1. A neurotransmitter comprising:
a buckminsterfullerene C60 bonded to a GABA, and either levodopa or dopamine.
2. The neurotransmitter of claim 1 further comprising a second and a third GABA and a second L-dopa bonded to the buckminsterfullerene C60.
3. The neurotransmitter of claim 1 or 2 wherein the buckminsterfullerene C60 bonded to the GABA
10 and either the levodopa or the dopamine is disposed within a zeolite.
4. A method of curing, treating, or prophylactically avoiding motor neuron dysfunction related to oligomeric alpha-synuclein plaque formation in Parkinson's disease, Lewy Body Disease, or Inflammatory Bowel Disease in a subject, comprising the step of:
administering to the subject an effective amount of a neurotransmitter including a
15 buckminsterfullerene C60 bonded to GABA and either levodopa or dopamine.
5. A method of curing, treating, or prophylactically avoiding motor neuron dysfunction related to oligomeric plaque formation in Alzheimer's disease or Amyotrophic Lateral Sclerosis (ALS) in a subject, comprising the step of:
administering to the subject an effective amount of a neurotransmitter including a
20 buckminsterfullerene C60 bonded to GABA and either levodopa or dopamine.
6. The method of claim 4 or 5 wherein administering the neurotransmitter comprises administering a composition containing a pharmaceutically acceptable carrier and the neurotransmitter.
7. The method of claim 6 wherein the composition comprises a tablet, capsule, pill, powder, granule, or a form suitable for injection.
- 25 8. The method of claim 6 wherein the pharmaceutically acceptable carrier comprises a zeolite.
9. The method of claim 4-7, or 8 wherein administering the neurotransmitter comprises administration by an intravenous, intramuscular, subcutaneous, intrathecal, intraperitoneal, topical, nasal, or oral route.
10. The method of claim 9 wherein an oral dosage comprises up to about 500 mg of the
30 neurotransmitter.
11. The method of claim 9 wherein administering the neurotransmitter comprises intramuscular, intravenous, or subcutaneous administration in an amount of from about 0.1 mg/Kg to about

5 mg/Kg.

12. The method of claim 9 wherein administering the neurotransmitter comprises administration by a nano aerosol, a vapor, a powder, a dust, or an aerosolized inhalant.
13. A method of making a prosthetic neurotransmitter comprising a C60 bonded to GABA and
5 either a levodopa or a dopamine, the method comprising:
bonding the GABA to the C60; and
bonding either the levodopa or the dopamine to the C60.
14. The method of claim 13 wherein bonding GABA to the C60 and bonding either the levodopa or the dopamine to the C60 are performed at no more than 40 °C.
- 10 15. The method of claim 13 or 14 wherein bonding the GABA to the C60 and bonding either the levodopa or the dopamine to the C60 is performed by reaction shear mixing.
16. The method of claim 13, 14, or 15 wherein bonding the GABA to the C60 and bonding either the levodopa or the dopamine to the C60 is performed in an oxygen-free environment.
17. The method of claim 13-15, or 16 wherein bonding the GABA to the C60 and bonding either
15 the levodopa or the dopamine to the C60 are performed together.
18. The method of claim 13-16 or 17 further comprising disposing the buckminsterfullerene C60 bonded to the GABA and either the levodopa or the dopamine within channels of a zeolite.
19. The method of claim 13-17 or 18 further comprising combining the buckminsterfullerene C60 bonded to the GABA and either the levodopa or the dopamine with a pharmaceutically
20 acceptable carrier.
20. The method of claim 13-18 or 19 further comprising adding the buckminsterfullerene C60 bonded to the GABA and either the levodopa or the dopamine to a mixture of glycerol and polypropylene glycol.
21. The method of claim 13-18 or 19 further comprising dissolving the buckminsterfullerene C60
25 bonded to the GABA and either the levodopa or the dopamine into a hyaluronic acid solution.