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(54) Title: METHOD FOR EVALUATING DRUG CANDIDATES

(57) Abstract: Two-dimensional and/or three-dimensional polymeric or extended solid arrays, such as arrays of a polydiacetylene backbone, are used to evaluate the organic/water partition coefficient and/or oral absorptivity and/or transcellular permeability of a compound by monitoring the change in the fluorescence or phosphorescence of the array upon exposure to the compound and comparing it to a known change in fluorescence or phosphorescence, respectively. The method can also be used to evaluate the ability of a compound to bind to a protein.

Method for Evaluating Drug Candidates

Description

Technical Field

The present invention relates to a method for evaluating possible drug candidates in the drug discovery process, using two and/or three-dimensional polymeric or extended solid arrays and preferably using polydiacetylene arrays. The arrays are exposed to the drug candidates and the fluorescence or phosphorescence of the arrays measured and compared to arrays exposed to standard compounds. The extent of emission of arrays exposed to drug candidates versus the emission of arrays exposed to known standards can be used to estimate and/or predict the organic/water partition coefficient of the drug candidates, and/or their oral absorption and/or their transcellular permeability. The arrays can also be used to evaluate the binding of drug candidates and other compounds to proteins, such human blood plasma proteins or other biological molecules.

Background of the Invention

Polydiacetylenes are conjugated polymers with backbones of alternating double and triple bonds formed from the 1,4-addition polymerization of 1,3-diacetylenes. Polydiacetylenes generally absorb well in the visible region of the spectrum, and hence are highly colored, ranging from blue to yellow. There has been intense interest in the non-linear optic properties of polydiacetylenes and extensive study has been made of both the solvo-chromic properties of solubilized polydiacetylenes and the thermo-chromic properties of polydiacetylene films and single crystals. It is well known that to form polydiacetylene, the diacetylene monomers must be in an ordered packing to allow the polymerization to occur. It seems to be generally accepted, though the inventors are not bound herein, that the packing of the side chains can affect the conjugation length of the backbone, and hence the chromic and emissive properties.

Diacetylene monomers have been used to form various ordered systems, including crystals, liquid crystals, liposomes and films that were then polymerized to form the polymer. Liposomes have been made from monomers with two diacetylene chains and polar head groups (such as phosphotidylcholines, and its analogues) and from monomers with single diacetylene chains. The liposomes can be polymerized with UV light or gamma-radiation. Monomer films have been formed by Langmuir Blodgett methods or cast from solvents and then also polymerized with UV light or gamma-radiation. The choice of monomer structure, conditions of liposome or film formation, and polymerization conditions all affect the conjugation length of the polydiacetylene backbone, and hence the color of the system. Upon heating, these polymerized systems can undergo a change in the effective conjugation length, from the longer length forms (blue and purple) to the shorter length forms (red and yellow). In the case of the packed polymer arrays that form the films and liposomes, it is generally accepted that changes in the environment that affect the organization and packing of the side chains coming off the conjugated backbone can affect the conjugation length and hence the chromic and electronic properties of the polymer.

These polydiacetylene films and liposomes have been suggested for chromogenic assays that depend upon color change (Charych et al, US Patent 6,001,556; Charych et al, US Patent 6,180,135; Charych et al, US Patent 6,080,423; Charych, US Patent 6,183,772; Charych et al, US Patent 6,022,748).

Color is an absorbance property; the colors we see are related to the wavelengths of light that the species is absorbing. For example if the species absorbs light primarily at 650 nm, we will see it as blue, while if it absorbs primarily at 550 nm, we will see it as red. Color arises from absorbance of light in the visible range. Most colored species are not fluorescent. If a colored species is fluorescent, it will normally appear one color, but when it is excited with the appropriate wavelength, it will glow with the color of the emitted light. For example, a fluorophore may look like an orange powder, but glow green under a UV lamp.

Polydiacetylenes can show fluorescence. However, their ability to fluoresce is dependent on the structural form and organization of the polymers (particularly the conjugation length and aggregation state), whether in solution, a film, or formed into liposomes or other three-dimensional structures.

It is known that polydiacetylene films have an intrinsic fluorescence when produced in the red or yellow form, and are not fluorescent (by conventional measurements) when the film is made in the blue form (Yasuda A. et al, Chem. Phys. Lett., 1993, 209(3), 281-286). This fluorescent property of the films has been used for microscopic imagining of film domains and defects.

More recently, it has been discovered that the change in polydiacetylene arrays from a non-fluorescent to a fluorescent state can be used for detecting an analyte by measure the emission of an array incorporating a ligand, receptor or substrate for the analyte. Furthermore the extent of this change can be magnified by incorporation of suitable fluorophores. These discoveries are described in patent applications PCT Patent Application WO 01/71317 A1 and US Patent Application S.N. 09/811,538 to Reppy et al., entitled "Method for detecting an Analyte by Fluorescence", and assigned to Analytical Biological Service Inc, the assignee of this application, disclosures of which are incorporated herein by reference.

Summary of Invention

The present invention provides a method to evaluate compounds by measuring their effect on the fluorescence or phosphorescence of two-dimensional and/or three-dimensional polymeric or extended solid arrays. More particularly, the present invention provides for the evaluation of compounds considered to be potential drug candidates, which comprises contacting the solution of the compound to be tested with a two-dimensional and/or three-dimensional array and preferably a two-dimensional and/or three-dimensional array comprising a polydiacetylene backbone. The change in fluorescence or phosphorescence of the array is measured or detected and compared to the change in fluorescence or phosphorescence, respectively, of the same arrays exposed to standard or reference compounds in solution. This comparison can be used to estimate the organic/water partition coefficient and lipophilicity and/or the likely oral absorption of the compound, and/or their transcellular permeability, and to evaluate the compound as a candidate for oral administration. The method can also be used to assess the binding of compounds to proteins or other macromolecules. The method can be used to rapidly screen thousands of compounds in an automated fashion in the drug discovery process.

Other objectives and advantages of the present invention will become readily apparent to those skilled in the art from the following detailed description, wherein only the preferred embodiments of the invention are shown and described, simply by way of illustration of the best mode contemplated of carrying out the invention. As will be realized, the invention is capable of other and different embodiments, and its several details are capable of modification in various apparent respects, without departing from the invention. Accordingly, the drawings and description are to be regarded as illustrative in nature and not as restrictive.

Summary of Drawings

Figures 1A, 1B and 1C illustrate a sequence of employing both a two dimensional array and a three dimensional array in a process according to the present invention.

Figures 2A and 2B, respectively show plots of change in emission between an initial reading and a final reading versus Log (P) (Figure 2A) and oral absorptivity (Figure 2B) for coatings exposed to test compounds.

Figure 3 is a plot of the Log (P) versus liposome emission for test compounds.

Figures 4A and 4B, respectively show plots of change in emission versus oral absorptivity graphs for liposomes with a test compound set (Figure 4A) and with only amine test compounds from the set (Figure 4B).

Figure 5 is a plot of the slope of change in emission over time of liposomes exposed to compounds plotted versus the % oral absorptivity of the compounds.

Best and Various Modes for Carrying Out Invention

In order to facilitate an understanding of the present invention the following definitions which are used herein are presented:

- Test compound: a chemical compound added to the test materials.
- Partition coefficient: ratio of the concentrations of a compound in equilibrium between two solvent phases, usually octanol and water or buffer. The concentration of the compound in the organic phase is the numerator of the ratio.
- Log(P): the logarithm (base 10) of the partition coefficient in octanol/water. This definition includes what are commonly referred to as "log(D)" values; i.e., partition coefficients of ionic compounds in octanol/water measured at various pH's.
- Oral absorptivity: portion of orally administered material that is found in the blood stream.

In addition to facilitate an understanding of the present invention, the discussion that follows will be predominantly directed to the fluorescent aspect of the present invention with the understanding, as will be apparent to those skilled in the art, that the discussion likewise applies to the phosphorescent aspect.

The preferred two-dimensional and three-dimensional arrays employed according to the present invention comprise a polydiacetylene backbone. The arrays are prepared by polymerization of precursor diacetylene arrays. The diacetylene precursor two and three-dimensional arrays may also contain species that are not diacetylenes.

The polydiacetylene backbones employed according to the present invention are known and need not be described herein in any detail and can range from being oligiomeric (from the reaction of three or more monomers) to polymeric. For example see U.S. Patent 6,001,556 to Charych et al, disclosure of which is incorporated herein by reference.

In this embodiment the polydiacetylene is formed from polymerizing a three-dimensional or two-dimensional array of diacetylenes. The array may also contain non-diacetylene species such as natural and unnatural phospholipids, cholesterol, steroids, gangliosides, sugars, lipids, proteins and other species including any of those found in cell membranes to increase the biomimetic character of the array. The array may also contain other non-diacetylene species and multiple diacetylene species.

Also, side chains with ordering head groups are typically bound to the polydiacetylene backbone. The head groups are typically polar. The polydiacetylene amphiphilic side chains also add to the biomimetic character of the array.

The preferred arrays are formed by polymerizing arrays of diacetylene monomers. The typical monomers are single or multi-tailed diacetylene surfactants with polar head groups. More typically used are single or bis-tailed diacetylene surfactants with polar head groups. The invention is not dependant on the use of any specific diacetylene surfactant, tail structure, or polar head group, but can be used with any diacetylene monomer that can be polymerized to give polydiacetylene in its non-fluorescent form or polydiacetylene in a fluorescent form that can converted to another fluorescent form with a different magnitude of emission.

Materials typically used as head groups in the present invention include, but are not limited to: carboxylic acids, carboxylate salts, amides, ethanol amide, amines, ammoniums, imines, imides, alcohols, carbamates, carbonates, thio-carbamates, hydrazides, hydrazones, phosphates, phosphonates, phosphoniums, thiols, sugars, sulfates, sulfonates, sulfonic acids, sulfonic amines, sulfonamides, amino acids (including glutamate and glutamine), peptides, nitro-functionalized moieties, carbohydrates, choline, ethylene glycol, oligiomeric ethylene glycol, poly(ethylene glycol), propylene glycol, oligiomeric propylene glycol, and poly(propylene glycol), and combinations thereof.

When the arrays of the present invention are to be secured or anchored to a support surface, the tails of the lipids can be selected to provide this function.

The two-dimensional and three-dimensional arrays of the present invention can be produced in any number of forms. Liposomes are one of the suitable three-dimensional array forms that can be produced. The liposomes can be formed in a number of different sizes and types. For instance, it is possible to form the liposomes as simple bi-layer structures. Additionally, they can be multi-layered in an onion type structure. Their size can also be varied. A suitable two-dimensional array form that can be produced is a film. The film can be mono-layered, bi-layered, or multi-layered.

Numerous other shapes can also be produced. Lamellae (Rhodes et al, Langmuir, 1994, 10, 267-275), hollow tubules and braids (Frankel et al, J. Am. Chem. Soc., 1994, 116, 10057-10069.), crystals, lyotropic and thermotropic liquid crystalline phases, gels and amorphous structures are among the other shapes that can be formed. When these assemblies are immobilized they can collectively form even larger constructs.

To facilitate a further understanding of the present invention, the preferred diacetylene arrays are discussed below, but it is understood that the various structures discussed can employ other arrays.

Diacetylene liposomes can be converted to tubules before polymerization by controlled cooling, concentration changes, or addition of ethanol. The tubules can be photopolymerized to give either the non-fluorescing form or the fluorescing form of polydiacetylene; either form can be used in assays with fluorescence monitoring. Polydiacetylene can also be formed as blue or red gels with a net-work structure of aggregated fibers. Polydiacetylenes can be used in the formation of composite materials, including layering with inorganic clays. Film arrays of diacetylenes or polydiacetylenes may be used in the free standing form, or supported on glass, ceramic, polymer, paper, metal, or other surfaces. The supports may be porous, including, but not limited to, nano and micro porous membranes. Diacetylene coatings may also be cast onto glass, ceramic polymer, paper, metal or other surfaces and photopolymerized to give the polydiacetylene array described above.

Diacetylene and polydiacetylene liposomes and films/coatings may be attached to, supported on, or absorbed in, solids, including, but not limited to: polymers such as polystyrene, polycarbonate, polyethylene, polypropylene, cellulose, cellulose esters, nylon and polyfluorocarbons such as Teflon®(polymers of tetrafluoroethylene) , perfluorinated ethylene-propylene copolymers, copolymers of tetrafluoroethylene and perfluoroalkoxy, copolymers of tetrafluoroethylene and ethylene, polymers of chlorotrifluoroethylene, and copolymers of chlorotrifluoroethylene and ethylene, polyvinylene difluoride, silicon chips, beads, filters and membranes, glass, gold, silica, sephadex, sepharose, porous or swelling solids such as polyacrylates and polyacetonitrile, and sol-gels. The solids listed here may also be further modified by chemical, or plasma gas, or other, treatment. In the case of diacetylene liposomes and film arrays, they are polymerized after incorporation with or attachment to the solid support.

An embodiment of solid supported polydiacetylenes is as an array on nano-porous membranes. Diacetylene liposomes can be forced in and onto membranes including 100, 200 and 400 nm membranes and photopolymerized to create a polydiacetylene coating. These coated membranes are stable at room temperature, in air, and exposed to light, for at least 12 months. The polydiacetylene array coating exhibits some resistance to abrasion. The polydiacetylene arrays can be converted from the non-fluorescent to the fluorescent form or from one fluorescent form to another fluorescent form with a different magnitude of emission or the fluorescent to the non-fluorescent form in response to environmental changes including exposure to a solution containing a test compound. In other words, the fluorescence of the arrays can be either decreased or increased by exposure to the test compound.

Nanoporous membranes are available in many materials, including: alumina, polyfluorocarbons such as Teflon® (polymers of tetrafluoroethylene) , perfluorinated ethylene-propylene copolymers, copolymers of tetrafluoroethylene and perfluoroalkoxy, copolymers of tetrafluoroethylene and ethylene, polymers of chlorotrifluoroethylene, and copolymers of chlorotrifluoroethylene and ethylene; nylon, polycarbonate, cellulose, cellulose esters, polyvinylene difluoride (PVDF), and glass and also in a variety of pore sizes. We envision using any of these membrane types with pore sizes typically up to about 600nm for preparing solid supported polydiacetylenes. The membrane surfaces may be further modified by chemical, or plasma gas, or other, treatment. Microtiter plates are available and can be made with nanoporous membranes for the well bottoms that can be precoated with the diacetylene or polydiacetylene arrays or coated with the diacetylenes arrays in situ and polymerized.

By way of example, the diacetylene two-dimensional and three-dimensional structures are photopolymerized with UV light, or gamma-radiation, to give organized polydiacetylenes with the longer conjugation lengths characterized by absorption maximum in the range of 500-800nm, preferably in the range 600-750nm, and a blue to purple color. The photopolymerization results in creating mainly the non-fluorescing form and therefore exhibiting low overall fluorescence relative to the background. The term "non-fluorescent form" as used herein also refers to these polymers which have low overall fluorescence exhibiting a fluorescent signal above 500 nm that is only about 1-3 times that of the background and less than that of the corresponding fluorescent form. The term "emission" as used herein refers to the intensity of fluorescence emitted at a wavelength or over a range of wavelengths. Typically the "non-fluorescent form" exhibits a fluorescent emission above 500 nm that is at least about 10% lower and more typically at least about 50% lower than that of the corresponding fluorescent form. Some diacetylene two-dimensional and three-dimensional arrays give polydiacetylene in the fluorescent forms upon photopolymerization; these may still be used in assays if interaction with a test compound converts the arrays to a fluorescing form having a different measurable emission that is either lower or higher than the original emission. Arrays may also be heated or exposed to other environmental changes, for example, a change in pH, to convert them to the fluorescent form before use in assays. In the application described here, the exposure of polydiacetylene arrays to compounds of higher log(P) can lead to a drop in intensity of the measured emission of the array versus the emission of arrays exposed to compounds of lower log(P) and to reference solutions.

The polydiacetylene fluorescence of the fluorescent form may be excited by light with wavelengths between 300 and 600nm, and consists of a broad fluorescence above 500nm with one or two maxima though the invention is not bound by these specifics.

The conditions that cause conversion to the fluorescent form also may cause chromic changes that appear to the eye as a blue to red shift, and can be quantified by measuring the UV/VIS absorption spectrum. Change in fluorescence and change in absorbance at specific wavelengths do not necessarily linearly correlate. It seems likely, though the inventors are not bound herein, that rise in the fluorescence emission can be a result of either a rise in the population of shorter conjugation length polydiacetylene backbones, or a decrease in the quenching longer conjugation length polydiacetylene backbones, or both. The relative change in fluorescence can be an order of magnitude or more, greater than the relative changes measured in the UV/VIS absorption spectrum upon this transformation. This means that fluorescence can provide a more sensitive measure of change in the liposomes than the direct chromic response. This increase in sensitivity makes the novel fluorescence detection method of use in many areas where colorimetric detection would simply not be sufficient. These include drug discovery assays. For films, the fluorescent/non-fluorescent properties of the polydiacetylenes can be used as a new detection method and would also provide increased sensitivity compared to colorimetric detection in immobilized sensing systems. Fluorescence detection also allows sensing platforms to use opaque supports, whereas colorimetric detection requires clear (e.g. quartz glass or UV/VIS-transparent plastic) supports.

It is possible to dilute the polymer array with other non-diacetylene surfactant species while still maintaining the liposome or film structure.

In a further embodiment of the invention, the arrays may incorporate other fluorescent or phosphorescent species. These fluorophores and phosphors may be organic, biological, inorganic or polymer compounds, complexes, or particles. The fluorophores or phosphors can enhance the magnitude of the change in the fluorescence or the phosphorescence, respectively, of the polydiacetylene arrays as they change from one fluorescent or phosphorescent form to another. The fluorescence or phosphorescence of the polydiacetylene fluorophores or phosphors, respectively, can also be monitored during this conversion, either as an internal standard if the fluorophore's fluorescence or phosphors's phosphorescence is not affected by changes in the polydiacetylene, or as an additional measure of the conversion when the fluorophore's fluorescence or phosphors's phosphorescence does change. In addition certain fluorophores can undergo excited state energy transfer processes that change the overall fluorescence of the array, and increase the quantum yield.

Many of the suitable fluorophores are lipophilic and are expected to incorporate into the alkyl region of the arrays, while others are polar or charged and are expected to end up in the head group region/aqueous interface, or in the water solution. The change in the arrays' fluorescence and the added fluorophore's fluorescence have been monitored as the arrays changed from the non-fluorescing to a fluorescing form, and from a fluorescing form to a less fluorescent form, under the action of heat, changes in pH or other environmental changes, chemical reactions, or during analyte detection. Various fluorophore additives had the effect of enhancing the percentage change in the array fluorescence response upon changing from the non-fluorescing form to the fluorescing form and from a fluorescing form to a less fluorescent form. The fluorescence of the added fluorophore was also monitored and also indicated the change in the liposome form from non-fluorescing to fluorescing, with some fluorophores showing an increase in their fluorescence and others a decrease.

For instance, the added fluorophores may optically and/or electronically interact with the polydiacetylene polymer. There are several ways that the fluorophores and the array can optically/electronically interact, including but not limited to the following means:

(1) By the fluorophore absorbing the fluorescence of the array or by the array absorbing the fluorescence of the fluorophore.

(2) By the fluorophore absorbing the excitation light, becoming excited and then transferring energy from its excited state to the polydiacetylene array causing it to fluoresce. This process is known as Resonance Energy Transfer (RET) and also as Fluorescence Resonance Energy Transfer (FRET). This RET process allows the polydiacetylene fluorescence to have the time decay properties of the fluorophore.

(3) By the polydiacetylene array, in its fluorescent form, absorbing the excitation light and then transferring the energy from its excited state to the fluorophore leading to the fluorophore fluorescing. This RET process can lead to an increase in the effective Stokes shift of the system and also increase the overall quantum yield.

(4) By the excited state of the fluorophore transferring an electron to the array or by the excited state of the array transferring an electron to the fluorophore. This process is known as Photoinduced Electron Transfer (PET).

(5) By the array absorbing the excitation light needed for fluorophore fluorescence or the fluorophore absorbing the excitation light needed for array fluorescence.

(6) By the fluorophore quenching the fluorescence of the array.

(7) By the array quenching the fluorescence of the fluorophore.

Addition of fluorophores or phosphors to the polydiacetylene array can make it possible to increase the extent of the change in fluorescence or phosphorescence, respectively of the array during an assay, thus increasing assay sensitivity; and also to monitor the fluorescence of the fluorophore or phosphorescence of the phosphor during the assay as a second measure of change in the polydiacetylene array caused by the analyte. The RET interaction between the fluorophore and the polydiacetylene array is discussed below.

Fluorescence Resonance Energy Transfer (RET):

While fluorescence monitoring using the intrinsic fluorescence of polydiacetylene is an improvement over absorbance (i.e. colorimetric) monitoring, polydiacetylene is a relatively weak fluorophore. Fluorescent polymers are often poor fluorophores because the conjugated chains can act as excited state traps and provide mechanisms for non-radiative relaxation of the excited state, leading to quenching of the fluorescence (Warta R. et al, J. Chem. Phys., 1988, 1, 95-99). It is theorized that the long conjugation lengths of blue polydiacetylene provide very effective trapping of the excited states which is why the blue form is effectively non-fluorescent (Morgan J. et al, Chem. Phys. Lett., 1992, 196, 455-461). Red and yellow polydiacetylene arrays actually contain mixtures of polymer chains of different conjugation lengths, and so it is quite likely that inter-chain quenching as well as intra-chain quenching, reduces the fluorescence of the shorter conjugation lengths.

The overall fluorescence of the arrays can be increased by incorporating certain fluorophores in the lipid regions, at the lipid/water interface and in the water solution itself (Reppy, M.A. "Signal Generation from Switchable Polydiacetylene Fluorescence", Mat. Res. Soc. Symp. Proc. 2002, 723, O5.9.1-O5.9.6). The RET process with certain fluorophores also significantly increases the Stokes shift from <100nm to >200nm. This is important when using plate readers to measure fluorescence as they often have less than optimal optics, or when reading the fluorescence of solids where there is usually high background from reflection of the excitation light off the surface. The incorporated fluorophores are directly excited by the wavelengths used to excite the polydiacetylene only to a minor extent or not at all, and so they do not make the non-fluorescent polydiacetylene arrays containing these fluorophores significantly fluorescent.

It is also possible for the fluorophores to transfer energy from their excited states to the fluorescent form of the array, causing it to fluoresce. A fluorophore can be excited at a wavelength that polydiacetylene is not excited by, and then transfer energy to the polydiacetylene leading to polydiacetylene fluorescence. This also may increase the effective Stokes shift of the overall polydiacetylene array. If the fluorophore has a long lifetime, for example certain terbium and europium compounds have long excited state decay times, the polydiacetylene fluorescence decay will have a similar lifetime. This makes it possible to perform time-resolved fluorescence (TRF) measurements using polydiacetylene arrays and common TRF readers (e.g., the Perkin Elmer/Wallach Victor2V). TRF has the advantage of giving a lower background as the background fluorescence usually has decayed before the measurement is made.

The fluorescence or phosphorescence can be read with any equipment known in the art for fluorescent or phosphorescent measurements, including, but not limited to, fluorometers with cuvette and fiber optic attachments, plate readers, hand held readers, fluorescence microscopes, CDC cameras, and by eye. This sensing method may be readily used in the multi-well plate formats of high-throughput screening.

The method of the present invention can be used in the field of drug discovery. Developers of pharmaceutical chemicals wish to test their compounds for biologically relevant physical properties such as solubility, lipophilicity, oral absorption, etc. In particular, developers are very interested in whether a drug, or potential drug, can be orally administered. If a drug can be orally administered it can be used much more widely and with much less expense than a corresponding drug that must be intravenously administered; hence the orally administered drug is likely to have a much larger market. Developers can evaluate the likelihood of their compounds having drug-like characteristics that would allow oral administration by using the so-called Lipinski rule of five. The Lipinski rule of five suggests that compounds with drug-like characteristics have fewer than five hydrogen bond donors, fewer than ten hydrogen bond acceptors, molecular weights lower than 500g/mol, and partition coefficients (log(P)) less than five. The first three criteria can be evaluated from the structure of the compounds; the last (i.e., log (P)) must either be measured directly or estimated through other means.

Measuring the equilibrium concentrations of compounds between octanol and water and taking the logs of the equilibrium constants directly determines partition coefficients. The direct measurement of partition coefficients requires significant amounts of sample and an analytical method for quantitating the concentrations of the species in the two phases. These measurements are not suitable for high throughput screening. Compounds have also been partitioned between buffer and phospholipid liposomes (Balon K. et al., Pharmaceutical Research, 1999, 16(6), 882-888), this also requires a separate analytical step for measuring the compound concentrations. Phospholipid liposomes are usually not stable for extended periods andor over wide pH ranges.

Animal and human studies to determine the oral absorption of compounds are expensive and time-consuming, and can only be used for a small number of compounds. A human intestinal derived cell line known as Caco-2 cells has been used for evaluation of compound oral absorptivity (Artursson P. and Karlsson J., Biochemical and Biophysical Research Communications, 1991, 175(3), 880-885). The cells are grown in a layer on filters (a process which takes over two weeks), test compound solutions in buffer are put over the cell layers and the permeability of the compounds through the cells is measured by analyzing (in a separate step) the concentration of the compound found on the other side of the cell layer. Currently it is not possible to efficiently evaluate large numbers of compounds (>50) in parallel with this method and the cells are not compatible with a wide pH range. Other cell lines have been used for similar testing. An alternative that uses 96-well filter plates, where the filters are impregnated with a mixture of hexadecane, has been proposed (Wohnsland F. and Faller B., Journal of Medicinal Chemistry, 2001, 44, 923-930); the passage of test compounds through the membrane is measured separately. Similarly, membrane supported lipid mixtures (PAMPA) are also used (Sugano K. et al., Journal of Biomolecular Screening, 2001, 6(3), 189-196). Chromatographic methods have been proposed where chromatographic columns are packed with silica functionalized with phospholipid ligands (Ong S. et al, Journal of Chromatography A., 1996, 728, 113-128) and the retention times of compounds are roughly correlated with oral absorptivity; again these methods are expensive and impractical to use for high throughput screening of thousands of compounds.

In all of these prior methods it is necessary to add a separate step for detecting whether the compounds have partitioned into, or passed through, the cells, membranes etc, by testing the solutions. This has been achieved by radiolabelling of the compounds, measurement of the UV-Vis (absorbance) spectra of the solutions, and potentiometric measurements. Radiolabelling is expensive and makes the compounds hazardous to handle; it is not practical for screening large numbers of compounds. UV-Vis measurements require that the compound have a chromophore, which is not always the case, and also determining where the compound has maximum absorbance. Potentiometric measurements are technically demanding and require specialized equipment. The present invention makes possible a one step detection or evaluation method that works with a wide range of compounds, does not require the test compounds to have chromophores or radiolabels, and can be performed using commercial equipment that is readily available.

The method of present invention makes it possible to predict (e.g., closely approximate) or estimate one or more of the physical properties of biological relevance of interest to drug manufacturers, at least as accurately as, or better than, these other methods discussed above that are used to estimate or predict these properties. The method of present invention is suitable for high-throughput screening of thousands of compounds in an automated fashion and can be used to evaluate a wide range of compounds.

One embodiment of the testing method of the present invention involves adding test compounds in aqueous or DMSO solutions to solutions of polymerized three-dimensional arrays (e.g. liposomes or tubules) in a 384-well or 96-well microtiter plate and measuring the emission of the wells after an incubation time ranging from minutes to hours. The emissions of the wells are compared to each other and to the emissions of wells containing control solutions. The emission of wells with characterized compounds is correlated to the log(P) of the compounds, and/or the transcellular permeability, and/or the oral absorption of the compounds and these correlations are used to gauge or estimate or predict the log(P) and/or the transcellular permeability and/or oral absorption of compounds for which these are unknown. The arrays may contain non-diacetylene species including, but not limited to: phospholipids, cholesterol, steroids, other lipids, proteins, cell membrane proteins, cell membrane components and fluorophores. The lipid species or other components may be synthesized or extracted from natural sources, such as cell membranes or bulk tissue. For example, the lipids and/or other components may be extracted from the cell membranes of intestinal lining cells. Lipids may be chosen to mimic the cell membranes of specific tissue types, e.g., brain, liver, fat, etc, or to mimic the membranes of various types of organisms, e.g., bacteria, yeast, etc. The arrays may be used over a pH range including, but not limited to, pHs of 2-10. The array solutions have been shown to be stable in storage over 10 months in some cases. The emission of the polydiacetylene arrays may drop relative to the control emission as they are exposed to compounds with increasing log(P)s and/or transcellular permeability and/or oral absorption. This decrease may be linear or have a "step-like" character with a sharp drop at a specific log(P) value. Alternatively, the emission of the arrays may rise relative to a reference as the log(P) values increase. The change in emission of the arrays appears to be dependent upon formulation; a compound could be screened with multiple array formulations to improve the precision of the estimation of the log(P) and/or transcellular permeability and/or oral absorption. Data analysis can be further refined by comparison of results for sets of compounds with structural and/or functional similarity. Analysis of the kinetic changes of the emission of arrays exposed to different compounds can also allow estimation of the log(P) and/or transcellular permeability and/or oral absorption. The emission or kinetic data gathered from the testing methods of the present invention can be analyzed alone, or can be integrated with computer or other models to predict physical properties of the test compounds of biological relevance.

Another embodiment of the testing method of the present invention involves adding test compounds in aqueous or DMSO solutions to polymerized two-dimensional arrays such as diacetylene arrays. The arrays may be on solid supports, or coated onto nanoporous filters, or free standing. The filters may be the filters of 96-well or 384-well or other well-count filter plates. The two-dimensional arrays may be arranged in a macro-array or may be in a continuous spread. The arrays may contain non-diacetylene species, including but not limited to: phospholipids, cholesterol, steroids, other lipids, proteins, cell membrane proteins, cell membrane components and fluorophores. The lipid species may be synthesized or extracted from natural sources, such as cell membranes. For example, the lipids and/or other components may be extracted from the cell membranes of intestinal lining cells. Lipids may be chosen to mimic the cell membranes of specific tissue types, e.g., brain, liver, fat, etc, or to mimic the membranes of various types of organisms, e.g., bacteria, yeast, etc. The arrays may be used over a pH range including, but not limited to, pHs of 2-10. The emissions of the arrays after or during exposure to the test compound solutions are measured. When the array is on a filter, in a further step the test compound solutions may be pulled or pushed through the coated filters and the emission read after that step. The emissions of arrays exposed to characterized compounds are correlated to the log(P) of the compounds, and/or the transcellular permeability, and/or the oral absorption of the compounds and these correlations are used to gauge or estimate or predict the log(P) and/or transcellular permeability and/or oral absorption of compounds for which these are unknown. The change in emission of the arrays appears to be dependent upon formulation; a compound could be screened with multiple array formulations to improve the precision of the estimation of the log(P) and/or transcellular permeability and/or oral absorption. Data analysis can be further refined by comparison of results for sets of compounds with structural and/or functional similarity. Analysis of the kinetic changes of the emission of arrays exposed to different compounds can also allow estimation of the log(P) and/or transcellular permeability and/or oral absorption. The emission or kinetic data gathered from the testing methods of the present invention can be analyzed alone, or can be integrated with computer or other models to predict physical properties of the test compounds of biological relevance.

Another embodiment of the testing method of the present invention of the present invention employs a polymerized two-dimensional diacetylene array as a thin mono, bi or multi-layer film. The passage of test compounds through this array as well as the change in emission of the array can be correlated to the absorption potential of the test compounds or to their partition coefficient. A test cell can be set up with the two-dimensional array separating a solution of three-dimensional arrays and a solution of the compound to be tested, as depicted in Figures 1A, 1B and 1C. Figure 1A depicts a cell containing a 2-D array film or coating and a 3-D array solution, while Figure 2B shows the cell with the addition of a test compound solution. The test compound partitions into and permeates the 2-D array passing into the 3-D array solution. The emissions of the 2-D and 3-D arrays are measured as illustrated in Figure 1C, and the measured values are used to estimate the partition coefficient and/or oral absorptivity and/or transcellular permeability of the test compound. Alternatively a polymerized array supported on a nanoporous membrane could be used in lieu of the film. One example of a test cell would be a coated filtration microtiter plate well. With appropriate instrumentation, such as bottom/top reading fluorescent plate readers, it is possible to measure the emission of both the three-dimensional array solution and two-dimensional array in situ. The emission of the two- the two-dimensional and the three-dimensional arrays are measured after a set time, or monitored at intervals, and correlated to the partition coefficient, transcellular permeability, and/or the oral absorptivity of the compound.

This testing method could be used in a further extension to determine compound binding to a protein or other macromolecules in solution. Suitable proteins include, but are not limited to, blood plasma proteins. In this embodiment of the proposed invention the compound is exposed to a protein and the mixture is then exposed to the polydiacetylene three-dimensional or two-dimensional array. The fluorescence of the array is compared to that of an array exposed to the compound alone, or compared to the fluorescence of the array exposed to another appropriate reference solution, to evaluate whether the compound has been bound by the protein.

Another embodiment of the testing method of the present invention employs a test in which the test compound and a protein or proteins or other macromolecules are placed in solution in a test cell in which this solution is separated from a three-dimensional array solution by a membrane of appropriate pore size, for example a dialysis membrane. The membrane prevents the protein or proteins or other macromolecules and any compound that may be bound to the protein, proteins, or macromolecule from coming into contact with the array solution, whereas unbound compound can pass through the membrane and contact the array solution. The emission of the three-dimensional arrays is measured after a set time, or monitored at intervals, to determine the binding of the test compound to the proteins or other macromolecules.

The following non-limiting examples are presented to further facilitate an understanding of the present invention.

In the following examples, unless otherwise stated, the diacetylene fatty acids were purchased from GFS or synthesized in-house. 10,12-Pentacosadiynoic acid (PCDA) was purchased; 6,8-docosadiynoic acid (DCDA), N-(2-Hydroxyethyl)-10,12-pentacosadiynamide (PCDA-EtOH) (Spevak et al, J. Am. Chem. Soc., 1993, 115, 1146-1147), (S)-N-(2-pentanedioic acid)-10,12-pentacosadiynamide (PCDA-Glu) (Cheng Q.; Stevens R. C., Langmuir, 1998, 14, 1974-1976) and mono 10,12-pentacosadiynyl phosphate (PCDA-PO4) (Hub H-H.; Hupfer B.; Koch H.; Ringsdorf H., Angew. Chem. Int. Ed. Engl., 1980, 19(11), 938-940) were synthesized. Acetylene compounds were purchased from GFS or Lancaster Synthesis. Reagents, anhydrous solvents, test compounds, cholesterol and buffer salts were obtained from Sigma-Aldrich and Fisher Scientific. Dimyristoyl phosphatidylcholine (DMPC), dioleoyol phosphatidylcholine (DOPC), dioleoyol phosphatidylethanolamine (DOPE), brain extract total lipids, and liver extract total lipids were purchased from Avanti Lipids. Organic fluorophores were obtained from Molecular Probes and Sigma-Aldrich; terbium and europium salts from Alfa Aesar. Solvents were obtained from Fisher Scientific in Optima grade unless otherwise specified. H2O was purified by ultra-filtration through a Millipore Milli-Q Plus system to a resistivity of 18.2 MΩ.

Probe sonication was achieved using an Imaging Products Sonic 300 V/T fitted with a micro-tip, with the power set to ~30%. Bath sonication was achieved using a Fisher Scientific FS140H sonicator, filled with water and heated to the appropriate temperature. A Kinematica PTA10 Polytron and a Cafrano BDC 1850 Teflon/glass homogenizer were used for dispersion and homogenization steps in the preparation of lipid extracts from cells. Sorvell RC5C and Beckman L7-35 centrifuges were used for centrifugation steps. Photopolymerization was achieved using a UV-oven capable of delivering calibrated energy doses of UV light around 254nm. Assay data were collected using both the SPEX Fluoromax-2 plate reader, and Wallach Victor2 and Victor2V readers. 384-well and 96-well black untreated polystyrene microtiter plates from Corning, and 96-well filter plates from Millipore were used in assays. 1H and 13C spectra were obtained by Acorn NMR, Livermore CA.

Natural lipid extracts were also obtained by processing pig intestinal lining cells and Caco-2 cells using an adaptation of literature methods. Procedures were performed keeping the solutions and mixtures on ice whenever possible; centrifuges were refrigerated to 4 °C. Cells were washed with 0.3M Mannitol/10mM HEPES/10mM TRIS buffer (pH 7.4, Buffer 1), homogenized in 0.3M Mannitol/1mM Dithiothreitol (DTT)/1mM TRIS/0.1mM Phenylmethylsulfonylfluoride (pH 7.0), and the suspension centrifuged at 500g for 5 minutes. The pellets were homogenized in 0.3M Mannitol/1mM DTT/1mM TRIS (pH 7.0) and centrifuged at 500g for 5 minutes. At this point the preparations for the two types of cells deviated.

For the pig intestinal lining cell preparation the pellets were discarded, the supernatants combined and made up in volume to 300mL, and 3 mL of 1mM $CaCl_2$ added dropwise. The mixture was stirred for 45 minutes and then centrifuged at 5,500g for 5 minutes. The supernatants were poured off and centrifuged at 38,000g for 40 minutes. The pellets were made up to 5 mL volume with Buffer 1 and homogenized 10 times at 2,000 RPM with a glass/Teflon homogenizer. The resuspended pellets were split into two 65mL centrifuge tubes and diluted to full tube volume with Buffer 1. These were centrifuged at 80,000g for 30 minutes.

For the Caco-2 cell preparation the pellets were made up to 5 mL volume with Buffer 1 and homogenized 10 times at 2,000 RPM with a glass/Teflon homogenizer. The resuspended pellets were split into two 65mL tubes and diluted to full tube volume with Buffer 1. These were centrifuged at 80,000g for 30 minutes.

For both preparations the final pellets were extracted with ice-cold, Ar-sparged, 1:1 $CHCl_3$/MeOH with vortexing followed by filtration. The solids were then sonicated in 1:1 $CHCl_3$/MeOH using a bath sonicator without heating, for 5 minutes, and filtered. This step was repeated. The combined filtrates had the solvent removed by rotary evaporation under partial pressure, without any heating of the flask. 4:1 benzene/ethanol was used to azeotropically remove water. The dried solids were partially redissolved in

1:1 $CHCl_3$/MeOH at 1.4 mg/mL; undissolved solids were removed by filtration. The filtrate solvent was removed as above and the solids dissolved in 1:1 $CHCl_3$/MeOH to 7 mg/mL (pig intestinal cell extracts) and 4 mg/mL (Caco-2 cell extracts). The solutions were stored at -20 °C.

Chloroform solutions of diacetylene surfactants were prepared by dissolving the appropriate amount of surfactant in chloroform and filtering the solution through 0.22 micron pore PTFE filters Diacetylene liposome solutions were prepared according to general methods presented in the literature (Hupfer B. et al, Chem. Phys. Lipids, 1983, 33, 355-374; Spevak et al, J. Am. Chem. Soc., 1993, 115, 1146-supplementary materials; Reichart A. et al. J. Am. Chem. Soc., 1995, 117, 829-supplementary materials) by drying organic solutions of the diacetylene surfactants together with organic solutions of any additives (e.g. phospholipids, lipids, cholesterol, and/or fluorophores), adding water or buffer to bring the combined materials to approximately 1mM overall, using probe sonication to disperse the materials, filtering the dispersion through a 0.8μm pore size cellulose acetate filter and chilling at 4 °C or 10 °C overnight. Coatings were prepared by using suction to filter liposome solutions through nano-porous membranes (50-200nm), or by extruding liposome solutions through nano-porous membranes. Liposomes and coatings were polymerized using a UV (~250nm) cross-linking oven capable of delivering a controlled dose. Compositions are given as molar percentages unless otherwise noted. Emission excitation is at 470nm unless otherwise noted.

Fluorophores included: 5-(((4-(4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)phenoxy)acetyl)amino)pentylamine hydrochloride (1); (E,E)-3,5-bis(4-phenyl-1,3-butadienyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (2); 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DIC-18(5)) (3), and 6-(((4,4-difluoro-5-(2-thienyl)-4-bora-3z,4z-diaza-s-indacene-3-yl)styryloxy)acetyl)aminohexanoic6-(((4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)styryloxy)acetyl)aminohexanoic acid sodium-4-sulfonate-2,3,5,6-tetrafluorophenyl ester (4).

Test compounds were taken from a set including: acebutolol, alprenolol, amiodarone, amoxicillin, ampicillin, ascorbic acid, atenolol, bifonazole, benzyl penicillin, caffeine, carbamazepine, ceftriazone, cimetidine, clofazimine, chlorpromazine, deferoxamine, desipramine, beta-estradiol, famotidine, flavone, foscarnet, fosformycin, furosemide, hydrochlorthiazide, hydrocortisone, ibuprofen, imipramine, isoniazide, kepone, ketoprofen, lactulose, mannitol, metoprolol tartrate, methotrexate, norfloxacin, ouabin, poly(ethylene oxide), pimozide, prednisone, pregnenolone, procainamide, propranolol, quinine, raffinose, ranitidine, salicylic acid, sulfasalazine, tetracycline, terbutaline, terfenadine, timolol maleate, triflupromazine, trifluoperazine. The compounds were dissolved in DMSO at 5.00mM and further diluted with DMSO or H_2O as appropriate.

Example 1:

Liposomes were prepared from 70% PCDA, 30% DMPC and from 70% PCDA, 30% DMPC with fluorophores 1 and 2 incorporated separately at 1 fluorophore:200 lipids, by probe sonication of the dried lipids in 2mM HEPES buffer at pH 7.4. The solution was filtered through a 0.8μ acetate filter, chilled at 10 °C overnight and photopolymerized with 0.2J/cm^2 of UV light at about 254nm. 4μL of liposome solution, 32μL of buffer (10mM sodium phosphate, pH 6.5) and 4μL of test compound solution (0.5mM in DMSO) were combined in triplicate wells of a black 384 well plate. The plate was shaken for one hour and the fluorescence of the wells measured and averaged as in Example 1 and compared to the reference wells. The liposomes exposed to compounds with partition coefficients (log(P)s) of 4 or lower have emissions similar to the reference liposomes; liposomes exposed to compounds with partition coefficients above 5 have emissions that are approximately 60% of the reference emissions. The sharp "step" in the emissions around partition coefficients of 5 makes these liposomes suitable for a simple screen to see if drug candidates will have a partition coefficient above or below 5.

Example 2:

Liposomes were prepared from 100% PCDA with fluorophores 1-3 in 2mM HEPES at pH 6.5 and polymerized with 400mJ/cm^2 UV as described above. 4μL of liposome solution, 32μL of buffer (10mM sodium phosphate, pH 6.5) and 4μL of test compound solution (0. 5mM in DMSO) were combined in triplicate wells of a black 384 well plate. The plate was shaken for one hour and the fluorescence of the wells measured and averaged as in Example 1 and compared to the reference wells. Plots of the partition coefficients of test compounds versus fluorescence of the liposomes with 1 and 2 incorporated at all three emission wavelengths showed a linear relationship with negative slopes. Liposomes with 3 incorporated showed no response to the test compounds with partition coefficients below 5.2 when compared to the reference. The emission at 675nm rose linearly with the rise in partition coefficients above 5.2; the emissions at 642nm and 572nm dropped, also in a linear fashion.

Example 3:

Liposomes were prepared from 57% DCDA, 26% DOPC, 7% DOPE, and 10% cholesterol, and from 57% DCDA, 26% DOPC, 7% DOPE, and 10% cholesterol with 2, 3, or 3 incorporated separately in 2mM HEPES buffer at pH 7.4 and photopolymerized with 0.4J/cm^2 of UV as described above. 10 μL of test compound solutions (0.5mM in DMSO), 80μL of buffer (2mM HEPES at pH 6.5) and 10μL of liposome solution were combined in triplicate samples in the wells of a black 96-well micro-titer plate. The emission of the wells at 675nm, 642nm and 572nm (excitation at 470nm) was read at 20 and 30 minutes, and averaged over triplicate wells. A roughly linear relationship is observed between the partition coefficients of the compounds and the emission above partition coefficient value of ~5; below a partition coefficient of ~5 the emission is more invariant.

Example 4:

Liposomes were prepared from 44% PCDA-EtOH/26% DOPC/7% DOPE/ 23% cholesterol and from 44% PCDA-EtOH/26% DOPC/7% DOPE/ 23% cholesterol with fluorophores 2, 3, and 4 incorporated as above. The liposomes were polymerized with 0.2J/cm2 of UV. 4μL of liposome solution, 32μL of buffer (10mM sodium phosphate, pH 6.5) and 4μL of test compound solution (0.5mM in DMSO) were combined in triplicate wells of a black 384 well plate. The plate was shaken intermittently over one hour and the fluorescence of the wells measured and averaged as in Example 1 and compared to the reference wells. For all three emission wavelengths and for all four formulations the emissions showed a sharp drop between partition coefficients of 4 and 5, similar to that seen in Example 1.

Polymerized liposomes from 44% PCDA-EtOH/26% DOPC/7% DOPE/ 23% cholesterol and from 44% PCDA-EtOH/26% DOPC/7% DOPE/ 23% cholesterol with fluorophores 2 and 4 incorporated, were also combined with buffer and test compound solutions (0.25mM in 5% DMSO/H_2O) in triplicate wells of a black 384 well plate as described above. The plate was shaken and the emissions read at 1h, 3h and 5h as above. The emission data for the plain liposomes (without fluorophore) and the liposomes with 4 incorporated showed a roughly linear drop in emission above log(P) ~3. The emission data for the plain liposomes also showed some correlation with the oral absorptivity of the compounds with the wells with the compound with the lowest oral absorptivity having a significantly higher emission than the other wells. These results were obtained with the test compounds at 25μM in the assay overall, versus the 50μM test compounds used in the first part of the example.

Example 5:

Liposomes were prepared from 44% PCDA-Glu/26% DOPC/7% DOPE/ 23% cholesterol and from 44% PCDA-EtOH/26% DOPC/7% DOPE/ 23% cholesterol with fluorophores 2, 3, and 4 incorporated as above. The liposomes were polymerized with 0.2J/cm^2 of UV. 4μL of liposome solution, 32μL of buffer (10mM sodium phosphate, pH 6.5) and 4μL of test compound solution (0.5mM in DMSO) were combined in triplicate wells of a black 384 well plate. The plate was shaken intermittently over one hour and the fluorescence of the wells measured, averaged as in Example 1 and compared to the reference wells. For all three emission wavelengths and for all four formulations the emissions showed a sharp drop between partition coefficients of 4 and 5, similar to that seen in Example 1 and Example 4.

Example 6:

Liposomes were prepared from 44% PCDA-Glu/26% DOPC/7% DOPE/ 23% cholesterol, from 44% PCDA-EtOH/26% DOPC/7% DOPE/ 23% cholesterol and from 44% PCDA-PO_4/26% DOPC/7% DOPE/ 23% cholesterol with fluorophore 4 incorporated as above. The liposomes were polymerized with 0.2J/cm^2 of UV. 4μL of liposome solution, 32μL of buffer (10mM sodium phosphate, pH 6.5) and 4μL of test compound solution (0.5mM in DMSO) were combined in triplicate wells of a black 384 well plate. A second plate was prepared with equivalent amounts using 10mM sodium phosphate at pH 7.4. The plates were shaken for one hour at 37 °C and the emissions of the wells measured (also at 37 °C) and averaged as in Example 1. The plates were kept at 37 °C and measured again at 3h, 5h and 20h. The data trends did not change significantly over time or with pH. For all three liposome formulations, at both pHs, the 572nm emission showed a strong drop in emission between log(P) values of 4 and 5; the 642nm and 675nm emissions showed a smaller drop.

Example 7:

Liposomes were prepared from 44% PCDA-Glu/26% DOPC/7% DOPE/ 23% cholesterol with fluorophore 2 incorporated (1) and from 44% PCDA-EtOH/26% DOPC/7% DOPE/ 23% cholesterol with fluorophore 4 incorporated (2) as above. A 96-well filter plate with 0.05μ pore mixed cellulose ester (MCE) filters from Millipore was pre-wetted by vacuum pulling 100-300uL of deionized water through the filters. Three columns were charged with 75μL of (1) per well; three columns were charged with 75μL of (2) per well and two columns were charged with 75μL of (1) and 75 μL of (2). The solutions were pulled through the filters under negative pressure using a Millipore vacuum manifold designed for plate filtering and a water aspirator. The plates were chilled at 4 °C for at least ½ hour and then exposed to 20mJ/cm^2 to polymerize the coatings, which formed even blue coatings. The plate was stored at 4 °C between preparation and use.

The drip directors on the plate bottom were sealed with water-proof tape, and 90μL of 10mM sodium phosphate, pH 6.5, were added to each well. After ~10m the emission of the wells at 675nm, 642nm and 572nm were measured (ex = 470nm). 10μL of seven test compounds at 0.5mM in DMSO were added to triplicate wells (duplicate wells for wells coated with (1) and (2) together). The plate was shaken at RT for one hour and the emission of the wells read. The plate was read again at 2.25h, 4h, and 20h. The tape was removed, the plate was then placed back on the Millipore vacuum manifold and negative pressure down to 30 Torr was applied. The solutions filtered very slowly; some wells showed no appreciable filtration. The plate was read again. The percent change in emission of the wells exposed to test compounds from the initial emission values in buffer was calculated and averaged over triplicate or duplicate wells. For the coatings made from (1) and (2) together the percent change showed a roughly linear relationship with log(P) with a negative slope; for the coatings made from (1) and (2) separately below log(P)~2 the percent change is fairly invariant and above log(P) of 2 there is a roughly linear decrease as the log(P) increases. The coatings from (1) and (2) together also show a trend of percent emission change with oral absorption; the compounds with oral absorptions of 90% or higher had percent emission changes of -28% to -39% while the compound with oral absorption of 1% had a percent emission change of -14%, all at 675nm. Similar trends were seen for emissions at 642nm and 572nm. The trends discussed above were seen at all time points and after the filtration step. The coatings made from (2) also showed a trend of percent emission change with Caco-2 permeability with a sharp drop in percent emission change between Caco-2 permeability of 21x10-6 and 1x10-6 cm/sec.

Example 8:

Liposomes were prepared from 44% PCDA-Glu/33%DMPC/23%cholesterol in 2mM HEPES at pH 7.4 as described above. Coatings were deposited on a pre-wetted 96-well filter plate onto 0.1μ MCE filters at 350 Torr, as described in Example 7 above, and photopolymerized with 10mJ/cm^2 of UV to form deep blue-purple coatings.

The coatings were exposed to 100μL of 10mM sodium phosphate at pH 6.5 (buffer) and shaken for 20 minutes. The buffer was then vacuum filtered through the wells at 300 Torr and the emission measured at 545nm (initial emission). Diluted test compounds were prepared by combining 30uL of 5mM test compound solution in DMSO with 2.97mL of buffer. 100μL of this was added to the wells and the plates shaken for 4 hours. The solutions were then filtered through the wells, at 300 Torr, and the emission measured again (final emission). Figure 2A shows the change between the initial and final emissions plotted versus the log(P) of the compounds for compounds with log(P) between 0-6, and Figure 2B shows the change between the initial and final emissions plotted versus the oral absorptivity of the compounds.

Example 9:

Liposomes were prepared from PCDA, PCDA-EtOH and PCDA-Glu with brain total lipid extracts or liver total lipid extracts, using a total of 8.8x10-7 moles diacetylene surfactant and 6.9x10-4 grams of extracted lipids, in 2mM HEPES at pH 7.4 for PCDA and PCDA-Glu based liposomes, and 2mM HEPES at pH 6.5 for PCDA-EtOH based liposomes, as described in previous examples. The liposomes were polymerized with 0.2J/cm^2 of UV; the PCDA/liver lipids liposomes did not polymerize and were discarded. 4μL of polymerized liposome solution, 32μL of buffer (10mM sodium phosphate, pH 6.5) and 4μL of test compound solution (at 0.05mM in DMSO) were combined in triplicate wells of a black 384 well plate. The plate was shaken intermittently over one hour and the fluorescence of the wells measured and averaged as in Example 1 and compared to the reference wells. The trends of change in emission relative to the reference versus log(P) were similar to those seen with the liposomes with DOPC/DOPE/cholesterol incorporated, as described in previous examples.

Example 10:

Liposomes were prepared from PCDA-EtOH and PCDA-Glu with lipids from Caco-2 cells and cholesterol, using 1.75 μmoles of diacetylene, 9.6x10-4 g lipid and 4.48 μmoles cholesterol, in 2mM HEPES at pH 6.5 and 7.4 respectively. The liposomes were polymerized with 0.2J/cm2 of UV, in a chilled 24-well plate on ice, and then heated at 70 °C for 5 minutes under an argon stream. 1.2 mL of liposomes were diluted with 10.4 mL of buffer (10mM sodium phosphate/150mM sodium chloride, pH 6.5). 58 μL of diluted liposomes and 2 μL of test compound solution (5mM in DMSO) were combined in the wells of a black 384-well plate and the fluorescence at 545nm was read every three minutes with shaking between readings. A plot of the 15 minute data of emission vs log

(P) of the compounds for log (P) from 0-8 was fitted to a line with a R^2 value of 0.76, as shown in Figure 3.

Example 11:

Liposomes were prepared from 44% PCDA-Glu/26% DOPC/7% DOPE/ 23% cholesterol in 2mM HEPES at pH 7.4, polymerized with 0.2J/cm^2 of UV as described for Example 6, then heated at 70 °C for 5 minutes under a stream of argon. 1.154mL of polymerized liposomes were combined with 8.840mL of 10mM sodium phosphate/150mM sodium chloride buffer at pH 6.5. 52 μL of the diluted liposomes were combined with 8 uL of test compound solutions (0.25mM in 5% DMSO/H_2O) in the wells of a 384-well black plate. The plate was shaken and fluorescence emission readings at 545nm were taken at intervals. Figures 4A and 4B show a plot of the change in the emission versus a reference solution versus oral absorptivity for all the compounds (Figure 4A), and for only the amine compounds (Figure 4B), at four hours.

Example 12:

Liposomes were prepared from PDA-EtOH (34.7 weight %), lipids extracted from swine duodenum mucosa cells (57.8 weight %), and cholesterol (8.5 weight %), in 2mM HEPES at pH 6.5, polymerized with 0.2J/cm^2 of UV, and heated at 70 °C for 5 minutes as described above. 6 μL of liposome solution, 8 μL of test compound solutions (0.25mM in 5% DMSO/H_2O), and 46 μL of 10 mM sodium phosphate buffer at pH 6.5 were combined in the wells of a black 384-well plate. The plate was shaken and the emission at 675nm, 642nm and 572nm were read at intervals over 99 hours; in between readings the plate was covered with a sealed cover. Comparison of plots of change in emission from the reference versus % oral absorptivity taken at different time points showed that measuring the emission any time from 15 minutes to 51 hours gave equivalent results. The plots showed a drop in the emission versus the reference for compounds with oral absorptions > 80%.

The slopes of the change in the emission versus time were calculated using data collected in the first hour. The slopes were plotted against the % oral absorption of the compounds as shown in Figure 5. This demonstrates an alternative way of processing the data for oral absorption prediction.

Example 13:

Liposomes were prepared from 44% PCDA-EtOH/26% DOPC/7% DOPE/ 23% cholesterol, as described in Example 4, with fluorophore **2** incorporated, and heated for 5 minutes at 70 °C. 85 μL of 0.278mg/mL solution of human serum albumin (HSA) in 10mM sodium phosphate, pH 7.4, were incubated at room temperature with 5 μL each of two test compounds (2mM in DMSO) and also with 5 μL DMSO as a reference, for 1.25 hours in the wells of a 384-well black plate, with shaking. 10 μL of the liposome solution were added to the mixed solutions, the plate was shaken further, and the emission read at intervals. One test compound had a relatively high affinity for HSA, and the averaged emissions of wells with HSA and the test compound together, and with the HSA alone, were similar, and differed from the averaged emissions of wells with the test compound alone. These results suggested that the test compound was bound to the HSA and not free to interact with the liposomes. The other compound had a low affinity for HSA, and the averaged emissions of the wells with the test compound and HSA and of the averaged emissions of wells with the test compound alone were similar. These results suggested that the test compound was free to interact with the liposomes.

The foregoing description of the invention illustrates and describes the present invention. Additionally, the disclosure shows and describes only the preferred embodiments of the invention but, as mentioned above, it is to be understood that the invention is capable of use in various other combinations, modifications, and environments and is capable of changes or modifications within the scope of the inventive concept as expressed herein, commensurate with the above teachings and/or the skill or knowledge of the relevant art. The embodiments described hereinabove are further intended to explain best modes known of practicing the invention and to enable others skilled in the art to utilize the invention in such, or other, embodiments and with the various modifications required by the particular applications or uses of the invention. Accordingly, the description is not intended to limit the invention to the form disclosed herein. Also, it is intended that the appended claims be construed to include alternative embodiments.

All publications and patent applications cited in this specification are herein incorporated by reference, and for any and all purposes, as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. In the case of inconsistencies the present disclosure will prevail.

CLAIMS

What is claimed is:

1. A method for evaluating at least one of the partition coefficient or oral absorption or cellular permeability of a compound, which comprises exposing a three-dimensional array of a polydiacetylene backbone or a two-dimensional array of a polydiacetylene backbone, or both, to the compound to be evaluated;

detecting the change in fluorescence or phosphorescence of the array,

comparing the change to a previously determined change in fluorescence or phosphorescence of the array.

2. The method of claim 1 wherein the array comprises a three-dimensional array in the form of a solution of liposomes or tubules.

3. The method of claim 1 wherein the polydiacetylene of the array is in the fluorescent form, and the decrease in fluorescence is measured.

4. The method of claim 1 wherein the increase in fluorescence is measured.

5. The method of any one of claims 1 to 4 wherein the three-dimensional array or a two-dimensional array further comprises a fluorophore and wherein the change in fluorescence of the polydiacetylene array is monitored.

6. The method of any one of claims 1 to 4 wherein the three-dimensional array or a two-dimensional array further comprises a fluorophore and wherein the change in fluorescence of the fluorophore is monitored.

7. The method of claim 1 wherein array does not contain a further fluorophore.

8. The method of claim 1 wherein the change in fluorescence is detected by exposure to light having wavelengths below 550 nm and measurement of the emission.

9. The method of claim 1 wherein the change in fluorescence is detected by exposure to light having wavelengths between 450 and 500 nm and measurement of the emission.

10. The method of claim 1 wherein the array comprises a two-dimensional array of a polydiacetylene backbone coated onto a solid support.

11. The method of claim 10 wherein the solid support is a porous membrane.

12. The method of claim 1 wherein the array is a two-dimensional array of a polydiacetylene backbone and is unsupported.

13. The method of claim 1 wherein the array comprises a two-dimensional array of a polydiacetylene backbone and which further comprises providing a filter or flow cell containing the array; and passing a solution of the compound through the filter or flow cell before or during the determining.

14. The method of claim 1 wherein the array is a two-dimensional array of a polydiacetylene backbone located on a non-porous support.

15. A method for evaluating the binding of a compound to a protein, which comprises exposing a three-dimensional array of a polydiacetylene backbone or a two-dimensional array of a polydiacetylene backbone, or both, to a solution of the compound to be evaluated post or during exposure to the protein;

detecting the change in fluorescence or phosphorescence of the array,

comparing the change to previously determined changes in fluorescence or phosphorescence.

16. The method of claim 15 wherein the array comprises a three-dimensional array in the form of a solution of liposomes or tubules.

17. The method of claim 15 wherein the polydiacetylene of the array is in the fluorescent form, and the decrease in fluorescence is measured.

18. The method of claim 15 wherein the increase in fluorescence is measured.

19. The method of any one of claims 15 to 18 wherein the three-dimensional array or a two-dimensional array further comprises a fluorophore and wherein the change in fluorescence of the polydiacetylene array is monitored.

20. The method of any one of claims 15 to 18 wherein the three-dimensional array or a two-dimensional array further comprises a fluorophore and wherein the change in fluorescence of the fluorophore is monitored.

21. The method of claim 15 wherein array does not contain a further fluorophore.

22. The method of claim 15 wherein the change in fluorescence is detected by exposure to light having wavelengths below 550 nm and measurement of the emission.

23. The method of claim 15 wherein the change in fluorescence is detected by exposure to light having wavelengths between 450 and 500 nm and measurement of the emission.

24. The method of claim 15 wherein the array comprises a two-dimensional array of a polydiacetylene backbone coated onto a solid support.

25. The method of claim 24 wherein the solid support is a porous membrane.

26. The method of claim 15 wherein the array comprises a two-dimensional array of a polydiacetylene backbone and is unsupported.

27. The method of claim 15 wherein the array comprises a two-dimensional array of a polydiacetylene backbone and which further comprises providing a filter or flow cell containing the array; and passing a solution of the compound through the filter or flow cell before or during the determining.

28. The method of claim 15 wherein the array comprises a two-dimensional array of a polydiacetylene backbone located on a non-porous support.

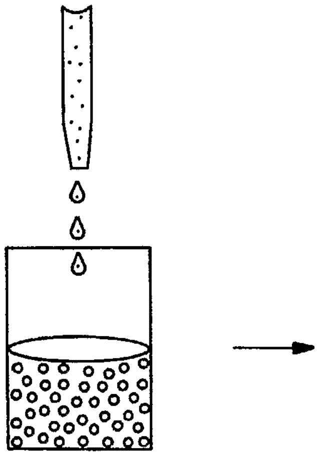

FIG. 1A

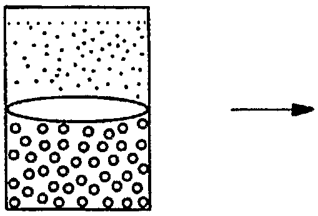

FIG. 1B

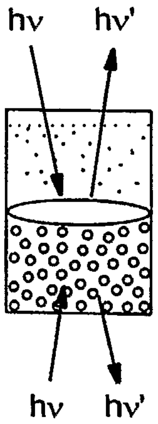

FIG. 1C

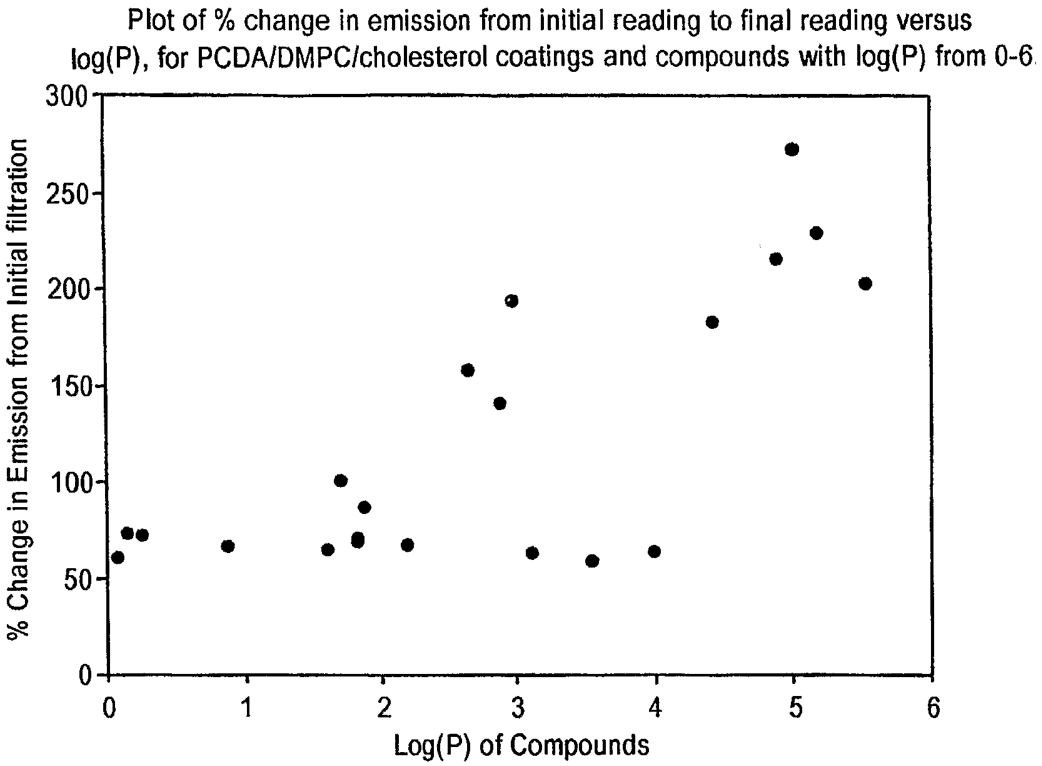
Plot of % change in emission from initial reading to final reading versus log(P), for PCDA/DMPC/cholesterol coatings and compounds with log(P) from 0-6
% Change in Emission from Initial filtration
300
250
200
150
100
50
0
0
1
2
3
4
5
6
Log(P) of Compounds

FIG. 2A

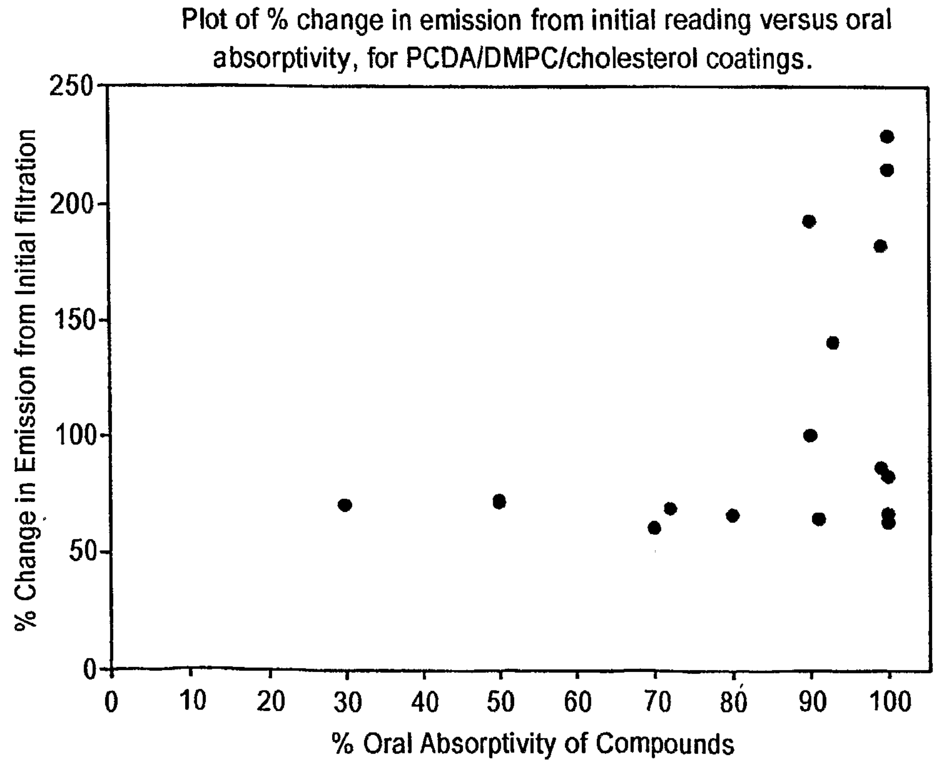
Plot of % change in emission from initial reading versus oral absorptivity, for PCDA/DMPC/cholesterol coatings.
% Change in Emission from Initial filtration
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
90
100
% Oral Absorptivity of Compounds

FIG. 2B

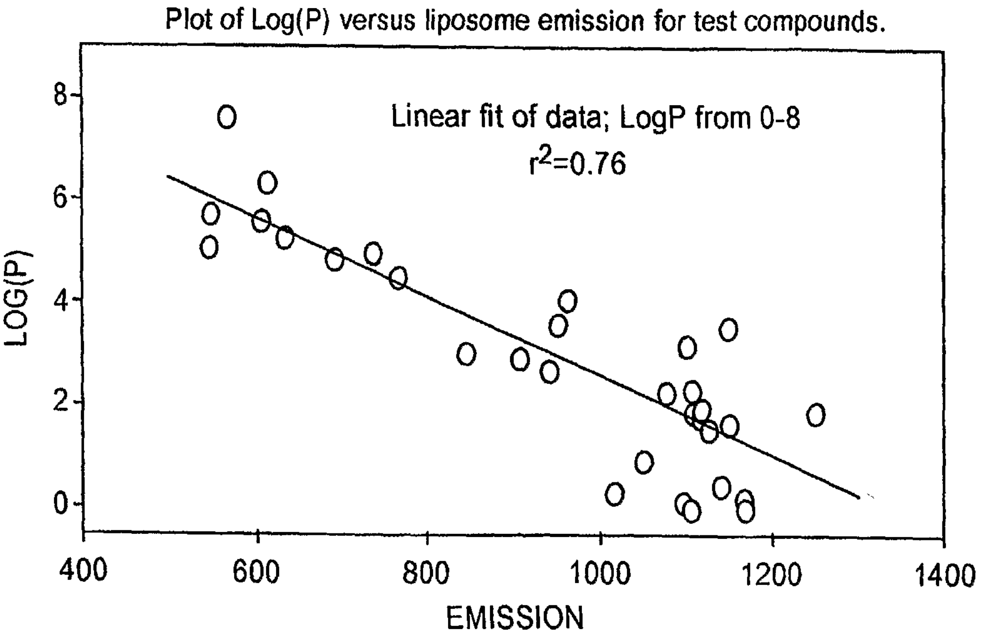
Plot of Log(P) versus liposome emission for test compounds.
Linear fit of data; LogP from 0-8
r²=0.76
LOG(P)
0
2
4
6
8
400
600
800
1000
1200
1400
EMISSION

FIG. 3

Change in emission versus oral absorptivity graphs for PCDA-Glu/DOPC/DOPE/cholesterol liposomes with a test compound set (A) and with only amine test compounds from the set (B).

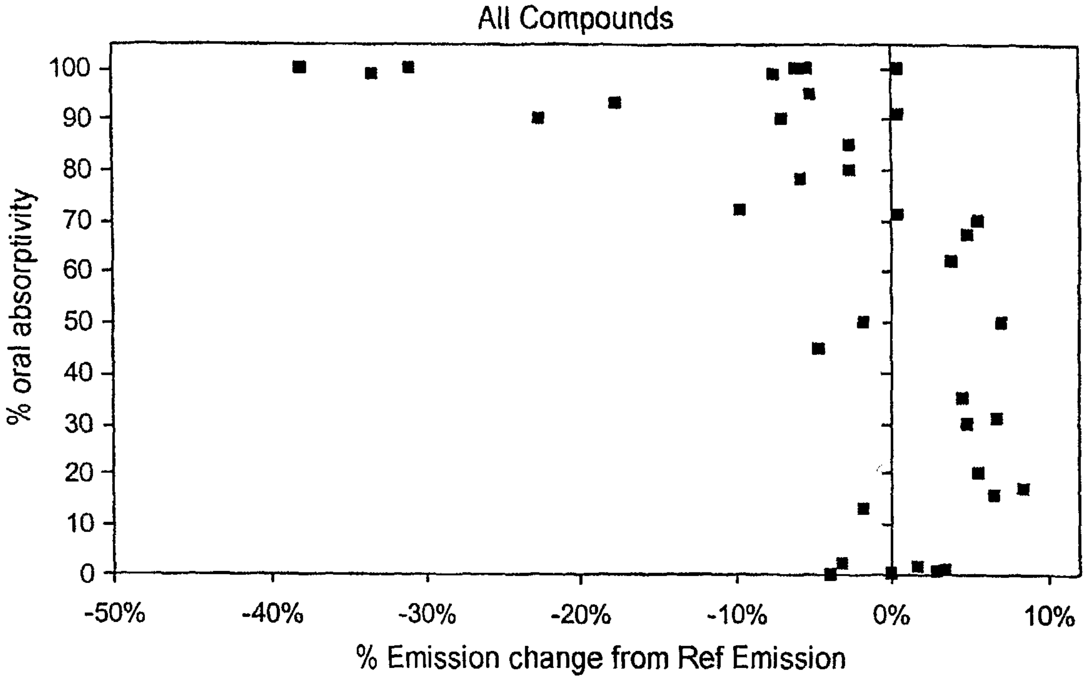

FIG. 4A

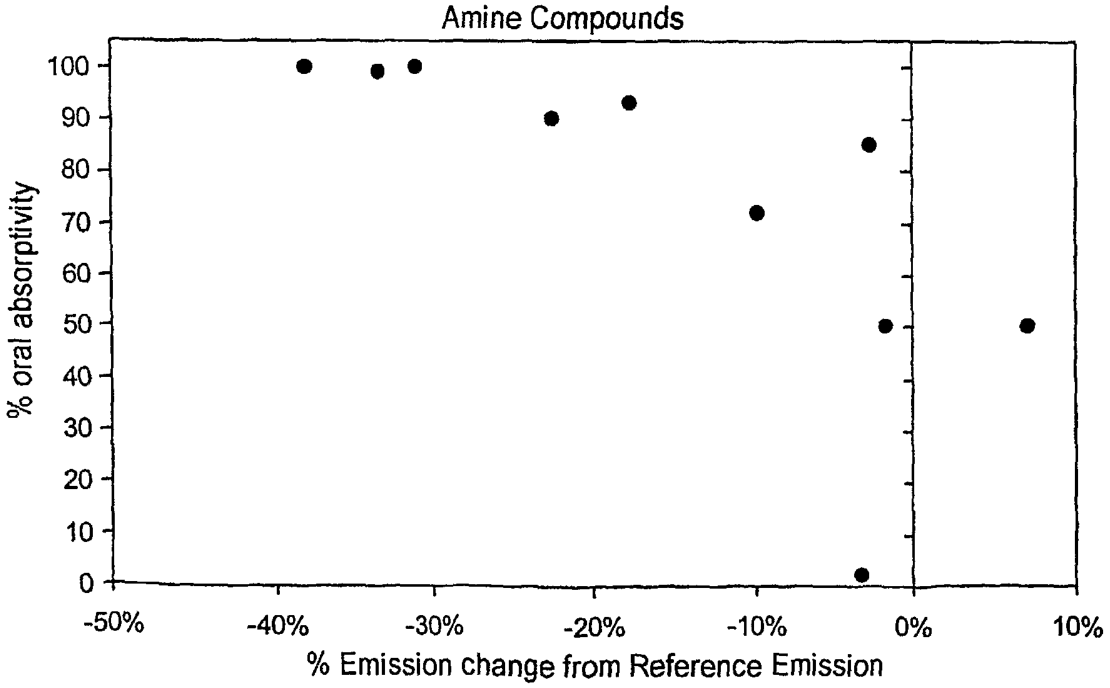

FIG. 4B

Slope of change in emission over time of liposomes exposed to compounds plotted versus the % oral absorptivity of the compounds.

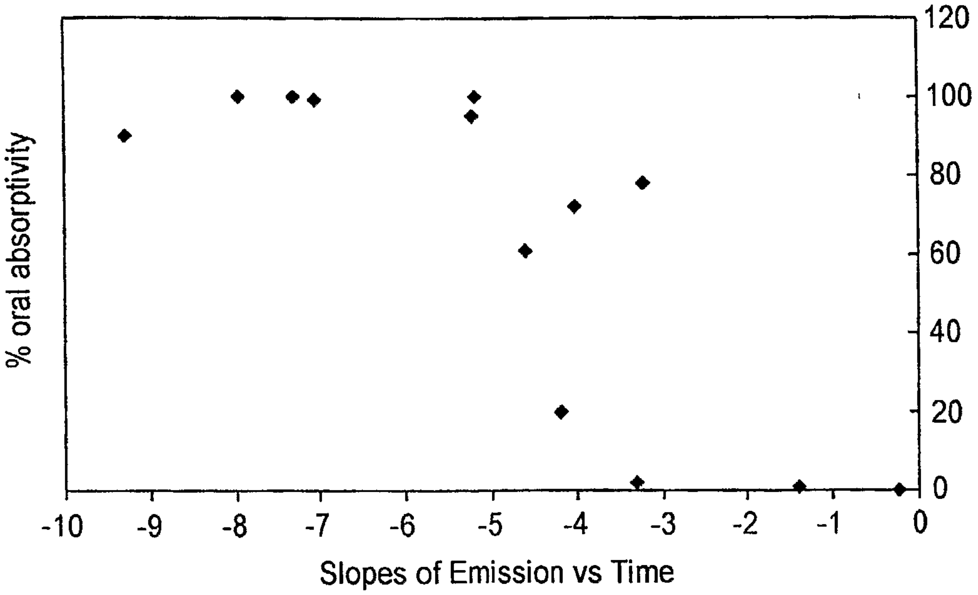

FIG. 5