

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 September 2007 (27.09.2007)

PCT

(10) International Publication Number
WO 2007/109096 A2

(51) International Patent Classification:
G06F 19/00 (2006.01)

(21) International Application Number:

PCT/US2007/006553

(22) International Filing Date: 15 March 2007 (15.03.2007)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/767,302 16 March 2006 (16.03.2006) US

PCT/US2007/03688

13 February 2007 (13.02.2007) US

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

(71) Applicant and

(72) Inventor: **THIESEN, Jack, H.** [US/US]; 219 Westchester Way, Easley, SC 29642 (US).

(74) Agent: **FARRELL, Martin**; 5002 Angler Lane, Greensboro, NC 27455 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,

Declaration under Rule 4.17:

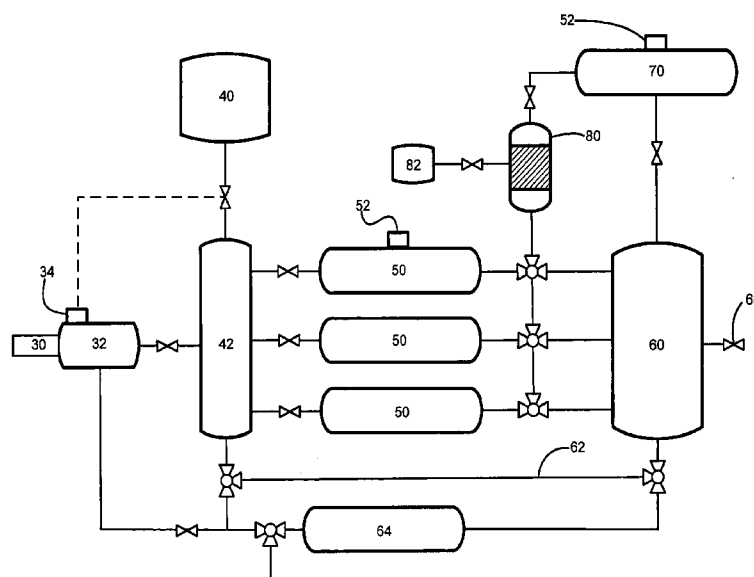
— of inventorship (Rule 4.17(iv))

Published:

— without international search report and to be republished upon receipt of that report

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

(54) Title: METHOD AND APPARATUS FOR CHEMICAL PROCESSES USING MOLECULAR IMPRINTS



(57) Abstract: An apparatus and method for chemical processes using molecular imprints includes a vessel having an interior surface formed with a plurality of molecular imprints, which may be of the same molecule or of different molecules and a sensor having imprints of a molecule formed on an active surface. A sample fluid is introduced into the vessel and the analyte molecules are adsorbed onto the imprinted surface and the sensor. The sensor indicates that analyte has been adsorbed. The imprinted surface may be used to separate the analyte and, optionally, to hold the analyte molecules for a reaction with components introduced to the vessel.

METHOD AND APPARATUS FOR CHEMICAL PROCESSES USING MOLECULAR IMPRINTS

[001] This application claims the benefit of US Provisional Patent Application No. 60/766,857, filed February 15, 2006, US Provisional Patent Application No. 60/767,302, filed March 16, 2006, and International Application No. PCT/US2007/03688, filed February 13, 2007.

[002] The present invention relates to a methods and apparatuses incorporating molecular imprints for separating, identifying, and introducing reagents in a chemical or biological process.

BACKGROUND OF THE INVENTION

[003] Molecular imprinting is a well known process for forming polymer structures that are selective for a particular molecule, called the print molecule or analyte molecule. In a typical imprinting synthesis reaction, a polymer matrix is formed around the print molecule and, after the print molecule is removed by washing or selective dissolution, the resulting structure is then an organic or inorganic matrix with physical imprints, i.e., complementary negative image imprints, of the print molecule that will attract print (analyte) molecules if exposed to them. Because the matrix is in fact a high fidelity mirror image of the source print molecule, there is an extraordinary degree of regio and stereo specificity creating a favorable environment for idiotypic reaction between the imprint and the print molecule.

[004] Molecular imprints have found use in such areas, among others, as sensor systems as described in US Patent No. 6,223,589 to Dickert et al., in synthesis of compounds having complementary structure to a print molecule, described in US Patent No. 6,127,154 to Mosbach et al., and for the preparation of artificial anti-idiotypic imprints described in US Patent No. 6,489,418 to Mosbach.

[005] A major disadvantage in the art is that molecular imprints are made directly from the print molecule, which severely limits the nature and quality of devices and apparatuses using molecular imprints for various tasks, such as separation and identification, for example.

SUMMARY OF THE INVENTION

[006] US Provisional Patent Application No. No. 60/767,302, filed March 16, 2006, and International Application No. PCT/US2007/03688, filed February 13, 2007, which are owned in common with the present application, and the contents of which are incorporated herein by reference, disclose methods for mass reproduction of molecular imprints. These disclosures also

describe methods for the formation of imprints of different molecules on the same substrate, which improves the flexibility of using imprints. Also disclosed are devices formed by such methods, for example, molecular sensors based on molecular imprints formed on mass sensitive sensor bodies.

[007] The present invention addresses deficiencies in analyzers, separators, and reactors by providing apparatuses and methods using multiple molecular imprints (complementary images of print molecules) made from molecular templates (surfaces having images identical to at least a portion of the print molecule) in new and advantageous ways. According to the invention, a separating and identifying apparatus includes a chamber having a wall or other surface bearing a plurality of imprints of an analyte molecule. At least one sensor, which could be a single sensor or a plurality of sensors, configured to sense the presence is disposed in the chamber, for example, on the wall or other surface. The apparatus includes means for introducing a sample to the chamber to expose the analytes to the imprints and an interrogator for obtaining a signal from the sensor.

[008] The present invention includes techniques to fabricate chemically sensitive surfaces and structures which can be used for sensors, analyzers, reactors, and other processing equipment, which surfaces and structures broadly fall into the class of imprinted idiotypic, antiidiotypic, and/or immunoassay templates. An immunoassay template is one in which an imprinted negative or mirror image of a molecule has been transferred and with which the molecule may subsequently react in an idiotypic fashion.

[009] Major advantages of invention, which will become apparent as the invention is described, include the ability to form the imprint of the print molecule on or in a large number of materials and the ability to place a molecular imprint with localized functionality very precisely onto other functional structures and devices.

[010] The present invention discloses methods and apparatuses for identifying, separating and manipulating biological and other molecules.

[011] In this case, because of the high degree of integration with the separation apparatus it is possible to make the interrogation process as transparent as possible to the demands of the chemical processes and in so doing provide the maximum amount of flexibility to the process designer. This means incorporating a sensor capable of making the measurement such as that cited and a convenient means of communication such as a wireless link between the sensor and

interrogator. In this case the antenna can both be directly integrated into the separation apparatus and the antenna for the receiver may be conveniently located with respect to the separation apparatus. Further, sensor feedback can be used to control the adsorption or desorption of previously adsorbed analytes, providing process control.

[012] According to the invention, an apparatus and method is provided for monitoring effluent streams, such as those associated with food processing, for the presence of pathogens or other undesirable molecules. For example, if one is concerned about the possible presence of E. coli in a vegetable or meat processing facility or a restaurant or about the presence of the H5N1 virus in a poultry processing facility, it is currently not possible to perform even the most cursory tests in such a way that so to be able to protect either the employees of such facilities or downstream consumers of food prepared and/or processed within them. Current tests, if they exist at all, typically require intrusive sample collection, sampling blood, vegetable matter, animal matter, or effluent. Such tests then often require the culturing and/or extraction and/or isolation/concentration of pathogens within the sample and then testing of the processed samples for the presence of the pathogens samples. All of this takes much time and effort, which delays or prevents rapid response to possible outbreaks.

[013] BRIEF DESCRIPTION OF THE DRAWINGS

[014] The present invention will be better understood by reference to the following discussion and when read in conjunction with the appropriate drawings in which like reference characters refer to like parts throughout the views, in which:

[015] Figure 1 is a schematic drawing of a separation and identification vessel in accordance with the invention; and

[016] Figure 2 is a schematic of an exemplary process apparatus in accordance with the invention.

[017] DETAILED DESCRIPTION OF THE INVENTION

[018] The invention is described here in terms of preferred embodiments, structures, and method steps, however, it will be understood that substitutions and equivalents as will be apparent to those of ordinary skill in the art may be used without departing from the scope of the invention as defined by the appended claims.

[019] The methods and devices disclosed and taught in International Application No. PCT/US2007/03688, are incorporated herein by reference. According to the those methods, a permanent imprinting template, having an identical image of the print molecule or at least a portion of the print molecule, is formed from the original print molecule and the template is used to form any desired number of imprints having negative images of the print molecule. . A print molecule as used here encompasses and molecule, protein, enzyme, hormone, biological material such as cells and viruses, or anything capable of forming a molecular imprint. Additionally, imprinting templates may be formed from combinations of print molecules, and accordingly, combinations of imprints may be formed on surfaces as desired.

[020] The permanent imprinting template may be used to print multiple copies of the print molecule image on a substrate or substrates, indeed any number of copies as long as the fidelity of the template does not deteriorate. Accordingly, a substrate may be a large or elongated sheet on which a multiplicity of imprints may be formed.

[021] According to the invention, the ability to print multiple copies of a print molecule on sheets, including combinations of imprint types, leads to a novel device, namely long columns or channels of imprints through which a solution may flow. A sheet of imprints is formed on a substrate having an appropriate surface as disclosed in the incorporated disclosures, using a printing template formed or mounted on a plate, a roller, or other device. As shown schematically in Figure 1, a sheet of imprints 10 is formed into or mounted in a tube or channel 14, with the imprinted surface 12 exposed to the interior of the tube. The channel 14 includes an inlet 16, which may be controlled by a valve, to introduce a sample flow to the interior and an outlet 18, which may also be controlled by a valve, to exhaust the flow. The sheet 10 may be positioned in a way to facilitate contact with a flow introduced into the channel 14, and may be rolled into a tube, corrugated, or arranged in some equivalent disposition. Alternatively, the sheet may be formed as a plurality of long, narrow sheets, which are mounted in a tube or channel to provide multiple passageways. The long column thus formed provides a great deal of surface area populated by imprints that can adsorb molecular, biological or other materials. Further, since different imprint types may be used, regions of the column may contain various forms and functional analytes.

[022] In addition, one or more sensors 20 may be integrated onto the surface 12 of the sheet or in the channel 14 in other advantageous locations. Sensors may determine when and to what extent the surfaces of the sheet 10 have become loaded with analyte molecules under study. The

sheet 10 and the channel 14 may further include devices to add energy to the sheet and/or the flow, such as ultrasonic energy sources, heaters, electrostatic pads, etc., to assist in adsorbing and desorbing the analyte molecules. The energy devices may be embedded in the sheet 10, attached to the sheet on a side opposite the imprinted surface 12, or otherwise disposed in the channel 14.

[023] The sensor 20 may advantageously be a mass sensitive sensor, for example, a wireless, surface acoustic wave resonant (SAWR) based sensor. Using the imprinting techniques of the aforementioned patent disclosures, it is possible to form and locate imprints precisely on the active surface of the sensor, thus improving sensitivity. It should be recognized that this resonant device may, alternatively, be a SAWD (SAW delay line device), FBAR, QMB (quartz mass balance), or a thin film bulk acoustic wave resonator (BAW). Further, imprints may be formed on any device with enough sensitivity to resolve very small amounts of adsorbed material such as cantilever beams, capacitive detectors and any other known to those skilled in the art. Reference is made to the aforementioned application PCT/US2007/03688 for a description of a mass sensitive sensor having an imprinted surface in accordance with the invention.

[024] Another type of sensor which may be used as part of a SAW sensor or independently is an RF impedance spectroscopy sensor. This sensor can operate with the electrode structure described above. This sensor functions by applying an RF signal between two electrodes and measuring the change in impedance, which can be generated by adsorbed molecules on the imprinted sheet or imprinted sensor surface. Another method for detecting adsorption of analytes is the use of light, where the reflected absorbance spectrum of an impinging light can be measured.

[025] An example of a wireless interrogator for the resonant sensor has an electronic circuit capable of generating RF energy at the resonant frequency of the sensor, which is transmitted to the sensor through an interrogation antenna. This energy is coupled into the sensor through an antenna and the coupled fraction of energy is stored in the resonator. After energizing the sensor, the interrogator stops transmitting and becomes a receiver as energy is reciprocally radiated back out of the sensor from the energy that has been stored. The interrogation electronics then sample the frequency of RF radiation of the device. The subsequent quantitative measurement of the reflecting frequency of the resonator provides a quantitative measurement of the adsorbed mass.

[026] The sensor may be further equipped with a heating element added to the structure. The heater can be used to selectively raise the temperature of the sensor and thus cause the analyte to desorb. By controlling heating and accordingly, temperature, through commands sent over a wireless link of the transmitter, it is possible to use the sensor indefinitely without maintenance or intervention. A further advantage of this invention is realized by analyzing the rate of adsorption/desorption as a function of device temperature at the imprint sites. For example, a differential measurement can be made to account for changes in the sensor response as a function of temperature. Then, as the temperature is increased, the mass change associated with desorption may be measured. However, there will be readsorption of the analyte molecules, and this rate is proportional to the concentration in the solution. Thus, by analyzing the steady state population of adsorbed molecules it is possible to determine the concentration of analyte molecules in the solution being tested. Another advantageous aspect of the present invention is that now the monitoring and control system can remotely control the process in real-time using feedback from the sensor, which can measure concentration and reaction states with minimum time delay and high local accuracy and precision.

[027] According to the invention, such a sensor can be integrated into a closed process providing the capability to selectively determine the concentration of reaction products.

[028] In addition, the sheet 10 and the sensor 20 may be provided with electrodes for generating an electric or magnetic field to align the molecules in the sample to facilitate adsorption. This is of particular advantage if the imprints on the sheet or sensor are formed in a process that includes aligning the print molecules with an electric or magnetic field, as described in the aforementioned patent applications.

[029] Further, when the sheet is provided with electrodes, an RF field generator may be provided to apply an RF field across the sheet for sensing the adsorption of analyte molecules by dielectric spectroscopy techniques. As analyte molecules are adsorbed onto the sheet, the impedance changes, which can be calibrated for amount of adsorbed analyte and differentiation between closely related adsorbates.

[030] Referring to Figure 2, an exemplary process apparatus for identification and/or separation of molecules is shown schematically. A sample inlet 30 is connected to an inlet reservoir 32 to introduce a sample to the apparatus. Samples may be provided in any convenient form, and for ease of handling, will typically be carried by a fluid, for example, a solvent that

does not alter the analyte molecule. An optional concentration test facility 34 is incorporated in the inlet reservoir 32 to test the introduced sample for concentration of particulates, analyte molecules, or other substances in the conveying fluid. This allows the system to dilute samples that have too much analyte and/or impurities. This also allows the system to separate the analyte from samples having too many impurities (for example, a sample for airborne pathogens in air collected in a dust storm). The concentration test facility 34 may, for example, include an opacity sensor or conductivity sensor. The concentration test facility 34 generates a dilution control signal which is sent to a valve controlling a flow of dilution fluid from a reservoir 40. The concentration test facility 34 controls the amount of dilution required for the system to operate properly, as explained below.

[031] Sample fluid flows from the inlet reservoir 32 into a dilution manifold 42. If the concentration test facility 34 senses a high concentration of material in the flow and generates a dilution signal, diluting fluid is added to the dilution manifold 42 from the dilution reservoir 40. The overall flow into the dilution manifold is the programmable ratio of flows from the various reservoirs.

[032] At least one sensor flow channel 50 is connected to receive the sample fluid, which may be diluted, from the dilution manifold 42. The at least one sensor flow channel 50 may be configured as the vessel shown and described in Figure 1, and may contain an imprinted surface and at least one imprinted sensor, where, imprinted here means having an imprint of the analyte molecule or molecules to be tested, analyzed, or separated.

[033] Analyte molecules are adsorbed onto the imprinted surface in the flow channels as the sample comes into contact with the imprinted surfaces. The analyte molecules will also be adsorbed by the at least one sensor incorporated in the channel 50. An interrogator 52 interrogates the various sensors and receives signals indicative of the analyte adsorbed by the sensors. Thus, a test solution introduced into the flow channels 50 is tested for the presence and concentration of analyte by analyzing the signal from the sensors incorporated in the walls of the flow channels 50 and keeping track of the volume of sample fluid introduced into the flow channels.

[034] Figure 2 illustrates three parallel sensor flow channels 50, each connected by a valved line to receive flow from the dilution manifold 42. The flow from the dilution manifold 42 may be accomplished by controllable valves. In many cases, one sensor flow channel may be

sufficient, however a plurality of sensor flow channels can provide several advantages, including that the sensor signals from each channel can be summed, improving the signal resolution by N where N is the number of sensor flow channels. This is advantageous in cases where the concentration is below the detection limit of the sensor in the flow channels. In this case, desorption of all adsorbed analyte molecules in the plurality of channels can be used to increase the concentration to provide a detectable signal to a downstream sensor.

[035] The sensor flow channels 50 are connected to an exhaust reservoir 60 by valved lines, where the sample fluid may accumulate after passing through the flow channels. The exhaust reservoir 60 can optionally be directly connected by a line 62 to the inlet reservoir 32 or the dilution manifold 42 to recycle the fluid. It may be desired to recycle the sample flow to the inlet reservoir when an extraction or separation process is being run, as will be explained below.

[036] Alternatively or in addition, the exhaust reservoir 60 is connected to a filter/deactivation vessel 64, where the exhaust fluid, which contains some analyte and impurities, may be filtered and, if necessary, biologically deactivated. Devices to provide ozone, UV light or another suitable means may be employed for deactivation. Filtering is accomplished using any known filtering means, taking into account the analyte and impurities to be removed from the fluid. The deactivated, filtered exhaust flow may then be disposed of or recycled to a mixing vessel for preparation of new sample flow, or returned to the dilution manifold 42 or the inlet reservoir 32 for further use in the process.

[037] The apparatus may also include a final flow channel 70 to collect and analyze the flow from the parallel flow channels 50. The parallel flow channels 50 can thus function as a separator and concentrator apparatus. For example, a sample including analyte and impurities and solvent is caused to flow into the channels 50. A mixture of the target analyte and impurities is adsorbed onto the imprinted surfaces. Heat and/or ultrasonic energy can be used to desorb the impurities, which will desorb at a lower energy than the adsorbed analyte, which is held deeper in the imprints. The desorbed impurities can be flushed away to the exhaust reservoir 60 by flowing a pure stream of dilution fluid or by operating the system in steady state. The control lines for sensor channel temperature and/or agitation control are not shown.

[038] After analyte has flowed across the sensor channels 50 for the desired time (after the majority of useful adsorption has taken place) and the impurities flushed off, the flow may be switched entirely to that of pure solvent, which may be delivered by the dilution reservoir 40 by

way of the dilution manifold 42. The clean solvent flow is directed through the flow channels 50. This flow may be used to flush away impurities creating a cleaner flow channel. The clean solvent may also be used as a carrier for analyte. In the latter case the analyte molecules adsorbed in the channels may be desorbed by using one of the previously disclosed means of desorption and released into the pure solvent. The solvent carrying the desorbed analyte is directed into the final flow channel 70, which can then analyze the now concentrated sample. By controlling the amount of conveying fluid and by ensuring all flow channels are desorbed, it is possible to provide a sample with fewer contaminants and having a much higher concentration. The separated sample will give a larger and more pure adsorption signal in the final sensor flow channel 70. The control lines for sensor channel temperature and/or agitation control are not shown. The flow goes from final sensor control channel 70 into the exhaust reservoir 60. If desired, this concentrated and purified flow may be drawn off the exhaust reservoir for other uses through an outlet 68.

[039] The apparatus may further include a reactor vessel 80 in which analyte, which may be separated and concentrated in the flow channels 50, is reacted with other molecules. Flow from the flow channels 50 is directed to the reactor vessel 80. A selected reactant flow is delivered from a supply reservoir 82 and allowed to react with the analyte in the reactor vessel 80. The reactor vessel 80 may include an imprinted surface to hold the analyte molecules in a particular orientation to facilitate or control the reaction with the supplied reactant molecules in the desired manner. This would be advantageous in DNA amplification, or other molecular manipulations, for example, as explained in more detail below. After processing in the reactor vessel 80, the products may be directed to the final flow channel 70 where they are analyzed. The products may then be collected in the exhaust reservoir 60, and drawn off by the outlet 68, if desired.

[040] As will be understood, the invention thus enables new processes and processing techniques for molecules, virus, pathogens, and other substances, as explained in the following examples.

[041] As described above in connection with Figure 2, the invention provides a method by which separation and analysis of a sample may be performed. According to the invention, the high degree of integration of the sensor device with the separation apparatus, that is, integrated on the imprinted surface or in close proximity, makes it possible to make the interrogation process as transparent as possible to the demands of the chemical processes and in so doing provide the maximum amount of flexibility to the process designer. The antenna can both be

directly integrated into the separation apparatus and the antenna for the receiver may be conveniently located with respect to the separation apparatus.

[042] Another example of the class of problem addressed by the current invention is found in the synthesis of pharmacological therapeutics. One critical aspect of the synthetic processing of pharmacological therapeutics is the necessity for cumbersome and expensive analyses in order to exclude undesirable byproducts and other impurities. Further, by their very nature many therapeutics require complex multistage reactions resulting in complex multi-component reactants/product solutions which are both difficult to separate and difficult to analyze. Complicating the assay of such solutions is the fact there can be molecules similar in nature but of an incorrect structure. Further complicating the analysis is the necessity for extremely hygienic conditions, especially if the therapeutic is for human consumption. In addition, interrupting running processes to perform analysis increases cost and complexity of the process. It is highly desirable to have no intervention and no interruptions which slow down such processes and increase the cost.

[043] The invention improves the state of the art for controlling a complex set of chemical reactions required, for example, in the production of therapeutics, although the process described below can be used for any complex chemical reaction. Briefly, this is accomplished by providing purified reagents, providing locally high concentrations of reagents, providing local conditions which promote the conversion reactants to products, providing a means for determining that the reaction has progressed to completion, and finally providing a means for separating the desired products from unwanted by-products and unconverted reactants.

[044] Example: Synthetic Reactions

[045] In this exemplary embodiment, a process is described using a separation and reactor apparatus substantially as disclosed above. Regions within the reactor are provided to separate the reactants from impurities, which are formed as separate regions on the walls of the reactor. Within these regions are embedded sensors capable of detecting that reagents have been adsorbed onto the walls of the reactor.

[046] The first step of the reaction process is to purify the reagents by adsorbing them out of the solution of reactants. The adsorption process may be aided by the use of ultrasonics and/or electric fields and/or heating or cooling as described in PCT/US2007/03688. The embedded sensor(s) are imprinted with the corresponding molecules for the particular region of the reactor

for detecting that the correct molecules have been adsorbed. The adsorption of molecules onto the imprinted sensors is nominally identical to the adsorption of identical molecules on the walls of the reactor. By tracking the relative change in frequency of the sensor as a function of time and by correlating frequency shift with the total molecular uptake it can be determined when sufficient numbers of molecules have been separated from the solution. This part of the process may take place in the flow channels 50 described above.

[047] After completion of reactant purification, the reactor may optionally be rinsed by one or more flushes with an appropriate fluid comprised of advantageous components which will remove unwanted impurities but leave the adsorbed reactants unaffected. Following the example apparatus of Figure 2, the separated reactants may then be desorbed and transported from the flow channels 50 to the reaction vessel 80. Alternatively, the next step may take place in the flow channels 50.

[048] After rinsing, a fluid comprised of advantageous components may be introduced into the reactor to facilitate conversion of the adsorbed reactants. Examples of advantageous components might be catalytic reagents, ionized reagents, oxidizing reagents, reducing reagents, a solution with a desired pH or just a fluid for component mixing to name a few possibilities.

[049] After introduction of the reaction promoting solution into the reactor, the reaction process itself may be initiated by desorption of reactants from the reactor walls. Reactants may be desorbed by local or general heating, the introduction of ultrasonic energy, application of an electric or magnetic field or the removal of such a field, or by any appropriate means known to those skilled in the art. The desorption of reactants may be en mass, individually, or in advantageous groupings. Further, the local application of heat, ultrasonics, electric and magnetic fields as well as the release of products may be such that the reaction progresses in an advantageous manner.

[050] As the reaction progresses, products of the reaction may be adsorbed onto selective surfaces in the manner previously disclosed for separation and extraction. Measurement of the shift in frequency of optionally embedded or inserted sensors with nominally identical adsorption surfaces may be made in a manner completely analogous to that described above. The ability to make such measurements provides means where the progress and ultimately, the completion of the reaction may be determined.

[051] When the reaction is complete, the reactor may be purged by the introduction of an

advantageously chosen fluid which removes the unreacted reactants and unwanted byproducts which were not adsorbed onto the reactor walls.

[052] After removal of all unwanted byproducts of the reactions as well unconverted reactants, the desired products may be collected. This may be accomplished by the physical removal of the imprint separation layer from the reactor or, as in this example, by the introduction of a final solution chosen to advantageously transport the products. The products themselves are desorbed from the wall into that advantageously chosen transport solution in a manner completely analogous and any means similar to those used to desorb reactants. Finally, it should be understood that these products may be used in subsequent reactions where the methods of separating, concentrating, purifying, positioning, etc., as described here can be used to facilitate complex, multistage reactions.

[053] Also, the system may include a self-test system including the capability to send warning messages to alert the user of the system to defective operation. Such a system would monitor the flows of the various components and reagents, the absorption rates in the different regions of the reactor vessel, and other factors to determine if the process is functioning as intended.

[054] As mentioned above, it is within the intent of this teaching that the walls of the reactor maybe removable and that after selected steps of a reaction an imprinted wall or walls may be moved from one location to another, or one vessel to another, and that the steps of the reaction detailed above might be comprised of movements of the imprinted walls as opposed to purging with rinsing solution. It should further be clear that any number of steps and permutations of the described steps as well various numbers of components and permutations of numbers of components to accomplish similar results for a wide variety of reactions are within the intent of this teaching.

[055] A method and apparatus for synthesis reaction may be improved by the capability of this invention for macro molecular manipulation based on precise and localized chemical modification. The methods and devices according to the invention provide a way that molecular imprints, and consequently, adsorbed molecules, may be located onto substrates with a high spatial accuracy for further manipulation. In principle, the accuracy is limited by the accuracy of the locating device which, for many stepping positioners on the market today, is less than one micron. This accuracy is expected to improve in the future. The ability to fix and adsorb molecules with such positional accuracy including that imprints of differing functionality may

be formed proximate to one another, and the functional device advantages disclosed herein provides the capability for very precise chemical modification of one or more macromolecules.

[056] The advantages of such a capability will become apparent through description of the following example of a stepwise reaction. For example, a molecule may be possessed of a particular structure under a given set of physical condition, i.e., the molecular structure can be controlled with temperature, pH, and other parameters. An example of such a molecule is a protein which folds itself differently depending upon the chemical environment. Such a molecule might be adsorbed onto an imprint as described above, where the imprint and the molecule have mirror image conformations. The molecule might be unfolded by locally heating or by a change in pH of the fluid environment, while it is held in place either through chemical functionality contained in the imprint or by a means such as an electric field, or a magnetic field (if a magnetic marker has been attached to the molecule in question). The unwrapped molecule may then be cleaved at a precise chemical location either through local chemistry (a release of an activated chemical) which may be initiated at the site and/or through heat and/or electric field, or through any other means known to those skilled in the art. After cleavage the molecule may have a new functionality attached to it by providing a flow with the new functionality into the reaction vessel. In this way, molecules may be precisely combined, they may be precisely tagged with a label such as a fluorescing molecule, they may be terminated and used as monomers for polymerization, they may have sections removed, the molecules themselves or their precisely cleaved fragments may be used in amplification reactions, or they may be attached to large macromolecules to add or modify functionality. In short, such a capability gives the molecular researcher a new set of tools whereby molecules may be precisely changed.

[057] A further improvement in chemical reactions is in the control offered by the present invention. Currently, most molecular modifications are run as statistical processes. Reactants and reagents are mixed and products are produced with certain statistical probabilities and at reaction rates governed by the local chemical environment as well as by the diffusion of reactants, reagents, and products through the mixture. Such statistical processes most often lead to a variety of products in addition to the desired products, especially when intermediate products and complex branched reaction pathways exist for intermediate products. The methods described above allow for very limited reaction pathways, high degree of control over the reactant's environment, and will therefore reduce or eliminate undesired reaction products. Further, as previously disclosed, the desired products may be separated from unwanted products

through adsorption of the desired products on imprinted surfaces. This is very important for reactions involving genes, gene splices, DNA, therapeutics, and the like since the ability to separate products allows for high purity and further manipulation, including the insertion of new functionality (as described above), replacement of defective functionality, the ability to control the length and structure of proteins replicated, and the initiation of replication processes. The invention further provides a means to create complex protein environments which might be used for cell differentiation, the realization of novel medications, the production of designer proteins and polymers, and more stable environments for cloning.

[058] Another advantageous improvement disclosed in this invention is the ability to form a much wider variety of self-assembled monolayers than is currently possible. In general, SAM structures are chemistry limited, typically capable of being formed only with certain chemistries which are sensitive or selective to Au and/or other metals. The molecular functionality which can be realized within the confines of these chemistries is severely limited. However, with the imprinting technology disclosed herein it is possible to form imprints of the functional ends of Langmuir Blodgett films, traditional SAMs, and, through the use of selective patterning as disclosed in PCT/US2007/03688, almost any imprint feature and/or combination of imprint features. Such features may then be populated with molecules whose features form the idiosyncrasy of the imprint. In general using these techniques a wide variety of two dimensional crystal or pseudo-crystals may be realized. Further this allows for the formation of a wide variety of SAMs with a much greater chemical variety.

[059] Example: Testing of Effluent Streams

[060] An apparatus according to the invention may be configured for environmental testing, such as the identification of carcinogens, pathogens, or other potentially harmful substances, without exposure of a testing professional to the hazardous environment. As previously described, an imprint surface may be prepared to adsorb many different molecules, including pathogens, from the same sample, thus streamlining the process and reducing the cost of conducting the test.

[061] The invention discloses a system and method for real time assays of bacteria, viral pathogens, prions, larger proteins, and chemicals. The system and method include means by which waste water, effluents from processing streams, and other flows for rinsing or cleaning food may be sampled. An apparatus as described in connection with Figure 1 may be connected

to receive and test a sample flow. The sample may be drawn from an effluent stream directly, from a continuous effluent spillover reservoir, or a periodically created spillover-reservoir formed by closing a valve at the base of the spillover-reservoir. Alternatively, a sample may be drawn from the fluid flow directly in a stream of washing fluid, the draw appropriately positioned where the stream will have collected contaminants. Samples may also be drawn from fluids extracted or collected from humans, such as sputum, saliva, urine, blood or others. It should be understood that such samples may advantageously be mixed with appropriate diluting and transport solvents.

[062] Samples of the effluent are tested using a sensor having a mass sensitive device with a surface adapted to adsorb the molecules being tested for, which can be prepared and used in the apparatus as described previously. Preferably, the sensor has a surface bearing imprints of the molecules of interest in testing. The sensor may be interrogated by means including wireless interrogation, direct (wired) interrogation and in fact any appropriate means for determining the state of the sensor. The sensor may be exposed to the sample by any suitable means such as, for example, inserting the sensors in the effluent stream, embedding the sensor in the walls of the vessel in which the sample is collected, or placing the sensors in a floating carrier in the effluent stream, sample, or reservoir. Interrogation may be by any appropriate and convenient means available adapted to determine the state of the sensor. Such a sampling device may correspond to the sample input 30 of Figure 2, where analysis is performed by any of the means and enabling methods disclosed herein.

[063] As previously disclosed, the sensor may be equipped or provided with means for desorbing analyte and contaminants from the surface of the sensor. For example, desorption may be aided by thermal energy with a heater embedded under the sensor, by an ultrasonic energy source embedded or placed proximate to the sensor, by flushing the surface with a solvent. Periodic desorption or flushing of the sensor surface makes the sensor capable of regular, periodic testing of the effluent.

[064] In addition, the apparatus includes means for flushing sampled effluent from the system to allow collecting fresh effluent for the next test. It is important to recognize that all the functions of the various components may be controlled via the use of an appropriate means of control, such as discrete logic circuits, state machines, and micro-controller as non-limiting examples. Such examples of system component and system control might have states set by receiving modulated RF over a wireless link, by modulated signals sent over wired cables such

as RS232. Finally there may also be a means whereby the system may test itself and send warning messages to alert the user of the system to defective operation.

[065] According to one embodiment of a method of the invention, a sample is introduced into a vessel having imprinted sensors and an imprinted surface, the sensors and surface being imprinted for at least one analyte. Analyte is adsorbed by the sensors, and the sensors are interrogated for the adsorption of the analyte. If the sample contains a high degree of other matter, which may be determined by a concentration test facility as disclosed above, the flow may be diluted and subsequently the analyte in the sample may be separated and concentrated as described. The separated and concentrated sample may then be introduced to a second vessel for reaction and/or analysis. By monitoring the volume of sample, the concentration of analyte may be determined. At the end of the test, the vessel or vessels may be flushed into a filter and deactivation vessel.

[066] One advantageous application of an apparatus of the invention is in a meat or produce packing plant to test the cleanliness of the meat or produce after a washing step. Another advantageous use is at a grocery or restaurant to test incoming shipments of fruit, vegetables or meats for pathogens, which could indicate how the shipment is to be handled (additional washing or decontamination) or whether it is accepted or rejected.

[067] Example: Testing of Air and Exhaust Streams

[068] The invention also provides a method and apparatus for continuously or periodically testing air for the presence of unwanted molecules or pathogens. The apparatus includes a device for capturing or producing an air sample or an air stream, and a means for introducing the captured air and/or air-stream into a fluid or fluid flow. For example, an air-pump can be arranged to draw test air and exhaust the air into a fluid reservoir, creating bubbles which pass through the fluid in the reservoir. The fluid reservoir could be a two stage reservoir, single stage reservoir, or a continuous stream of fluid.

[069] An alternative way of collecting and introducing airborne samples is through electrostatic adsorption onto plates where a voltage has been applied between the plates. The plates may then be placed into a solvent bath where desorption of the collected matter takes place. This may be aided by the introduction of temperature, ultrasonic, or other chemical processes known to those skilled in the art.

[070] Similar to the system described in connection with Figure 2, the system also contains a sensor to perform the assay, formed as described above to be sensitive to the molecules of interest and an interrogator for communicating with the sensor, such as an RF link, or a serial communications link. The system may also contain a heater, ultrasound generator, or other device for desorbing adsorbed analyte molecules as well as cleaning debris from the sensor. A filter and deactivation vessel may be connected to receive exhausted test fluid, in which the fluid may be sterilized and/or filtered and returned to the reservoir.

[071] The system may be made self-sustaining, by including a device for collecting water from rain and/or having water replenished by a service line for the fluid reservoir. Power could be provided by a generating device activated by ambient vibrations, solar power, wind power, or other device. Alternatively the power might be provided by batteries and/or building power. Finally there may also be a means whereby the system may test itself and send warning messages to alert the user of the system to defective operation.

[072] A test method for environmental air or an exhaust air stream begins with the step of introducing the air sample into an appropriate fluid, which might be any suitable fluid such as water, in a test vessel. Alternatively, the air sample may be introduced into a fluid in a secondary reservoir followed by movement of the fluid to a test vessel from the secondary reservoir via valves and pressure from the air pump. The sample is then tested using a sensor capable of preferentially adsorbing the analyte in question. The sensor is then interrogated where any adsorbed pathogen and/or analyte will produce a measurable change in the sensor operational parameters. Such operation will require time differential measurement, which can be done by using two sensors, one with imprints formed on a polymer surface and the second, identical sensor having a layer of the same polymer, but having no imprints. By comparing the time integrated signals of the two sensor, very sensitive and nearly time and condition invariant measurements may be made. In this way, the measurable change is determined and communicated via the interrogation link. Further, there may be need for calibration to account for discrepancies between the disposed pair of sensors. Calibration may be performed in a test apparatus or may be included after manufacturing and be included advantageously with the sensor pair and stored by any convenient interrogation means. Such interrogations may be by direct contact or wirelessly by means such as an RFID. A further advantage of the present invention is the use of amplification as previously disclosed to increase the concentration and therefore the detectibility of highly dilute and dispersed samples such as often found in airborne

contamination. The interrogation may take place using the provided means of communication.

[073] It should be understood that a gas may also be sampled and processed directly without introducing the analyte into a liquid by direct adsorption from a carrier gas onto sensors prepared as previously disclosed and by means previously disclosed. Those skilled in the art will recognize that the fluid described in the methods and apparatuses herein may be a gas or gases.

[074] After the test is performed, the temperature at the sensor surface may be increased to desorb the analyte and any remaining debris. The sensor reservoir may then be flushed and the sample fluid purified and filtered and returned back into the main reservoir.

[075] Another embodiment of the present invention is a system for the identification of pathogens or other molecules that allows self-directed testing. This system may be used, for example, by a person who may be infected with a pathogen or by a homeowner to test well water for contamination by pathogens or pollutants. The description below is in the context of pathogen testing, but should be understood to be applicable to any self-directed testing. The system includes highly selective sensors as previously described in conjunction with a device for dispersing a sample including suspected pathogens into a solution for assay by the sensors and a compatible means for quantifying the presence of such pathogens. Consequently, the amount of high-risk and high-cost intervention by medical and laboratory professionals may be substantially reduced. This invention further provides an advantageous way to make a determination of the presence of the pathogen and report the result of the determination at a greatly reduced expense, thereby reducing a portion of the burden of ever increasing healthcare costs.

[076] In this embodiment of the present invention, a variation of the apparatus of Figure 2, in portable form, may be adapted for this test system. The test system includes a sensor device, as previously described, in the form of a test coupon including a sensor capable of adsorbing the suspected pathogen and an associated interrogator to identify and quantify the pathogen. The sensor is formed with molecular imprints, as described in PCT/US2007/03688, of the suspected pathogen or pathogens for assay using the techniques disclosed herein. The test coupon further serves to associate the test with the person and includes some identifying indicia and/or device such as an RFID tag. The test coupon and sensor form an imprinted surface capable of being inserted and removed from different test vessels for the process here described. If the nature of the solution in which the sample is collected is such that the pathogens are denatured, the

molecular forms imprinted onto the sensor must be identically denatured. Alternatively, the molecular imprints used to test for a pathogen may be included in the apparatus, in which case the coupon serves only to associate the sample with the person from whom the sample was taken.

[077] A first step is sample introduction, which is the collecting of a sample containing the suspected pathogen from the subject person and introducing that sample to the system. One example of a sample introduction device is a means for bubbling a gas containing the suspected pathogen through a liquid, for example, the subject person's expired breath, through an appropriately chosen solution in which the sample dissolves. For example, such a device may include a tube having a free end in which the subject blows in expired air, and a second end immersed in the test solution. Alternatively the sampled may be tested directly from the gas phase as previously indicated.

[078] Another alternative introduction device is a device for mixing blood or a saliva into a test solution. For example, a sample could be collected from a swab of saliva or by having the person gargle a solution, such as a very dilute saline or sugar and water solution, and expectorate the gargled solution into a receptacle. The test solution and expectorated sample are mixed so that the pathogens collected in the expectorated sample may be further distributed to improve adsorption by the test coupon sensor surface. Dispersal of the sample in the solution may be by stirring, shaking, heating or any other means of mixing known to those skilled in the art.

[079] After dispersal of the sample in the test solution, the solution is exposed to the molecular imprint surface for adsorption, for example, by inserting the coupon into the receptacle. The coupon surface may optionally be provided with a means to promote adsorption such as electric and/or magnetic fields, ultrasonic energy sources and/or heating and cooling apparatus as described above, which may be part of the coupon or integrated into the receptacle. The use of energy sources promotes adsorption of analyte molecules when the energy is at a level below the desorption energy threshold of analyte molecules into imprints but greater than the desorption energy threshold of unwanted species on the imprint surface.

[080] The imprint surface, which may be formed as part of the test coupon or on another surface in the receptacle, is then analyzed using any of the means previously disclosed to determine the presence of the suspected pathogen molecule by an appropriately programmed interrogator. The interrogator provides a report of the results of the analysis. The coupon may

include imprints for one or several pathogens, for example, the common strains E-coli, or combinations of E-coli and salmonella and other food borne pathogens. Another example is rhinovirus or bacterial infections for which a coupon could include the major strains of rhinovirus and/or common bacteria, for example, streptococcus. By disposing imprints of various pathogens responsible for similar symptoms on a single coupon, utility of the coupon is enhanced and the probability of false report is reduced. The joint probability of a false negative and a false positive is smaller than the probability of either. In general, it should be understood that the imprints could take the form of any pathogen, and the invention is not limited by the description of the examples herein. The report may be in the simple form a positive or negative indication, or may include more information if the coupon is adapted to test for more than one pathogen.

[081] If the coupon is for identification only, the coupon would be placed where it could be interrogated for the identification information by the interrogation device, which information is used in the report. Alternatively, the report could be written onto the RFID tag on the coupon.

[082] After analysis the receptacle containing the test solution(s) may then be evacuated using a pump or other appropriate device, and the receptacle and the test coupon may be sterilized. The evacuated solution, receptacle, and coupon may be sterilized with UV radiation, heat, ozonated water, or any number of solutions capable of killing the pathogen. The surface containing the imprints may be restored by desorbing the adsorbed print molecules using heat, ultrasonic energy or any suitable means for initiating desorption from the imprinted surface.

[083] A device for self-directed testing may be portable and rented or sold to the user. Alternatively, the device may include a portable receptacle in which the sample is collected and dispersed and the coupon inserted. The portable receptacle may be returned to the interrogator part for analysis. As contemplated, the system may include means for payment to further facilitate self-directed use of the system. Payment may optionally be provided by the user in order to receive the test coupon, to receive the receptacle for introducing the sample into the test coupon, or to activate the analytical equipment. A known device for accepting and processing payment, for example, including a card swipe, may advantageously be included. Acceptable forms of payment may include credit cards, plan-provider drug cards, plan provider insurance cards, cash, or any other suitable means. Payment may be made at the device adapted to accept the payment and record the transaction, and then release a coupon to the payor and/or further

active the system to perform the analysis and deliver the report.

[084] A system and method according to the invention for determining the presence of pathogens is an advantageous advance in the art especially when applied to virulent airborne pathogens such as the SARS virus, communicable bacteria, sexually transmitted diseases, both bacterial and viral, and blood borne pathogens. It will be recognized that sample introduction could be obtained from expired breath, saliva, blood, scabs, pus, or other body fluids. For example, in the case of a medical emergency such as the outbreak of a virulent pathogen, a device could be provided that is capable of identifying several suspected pathogens. Further facilitating the use, the device may easily be made light and portable. As will be understood, the device may be interrogated without direct physical contact between the possibly infected person and the test personnel. In addition, there may also be a means whereby the system may test itself and send warning messages to alert the user of the system to defective operation.

[085] Such a system and method may advantageously be applied to ensure the safety of the blood supply, which can be done prior to or after collection from donors, or prior to administering blood to a patient. The method includes withdrawing a small portion of the blood in question and possibly mixing the sample with an appropriate dilution agent to reduce viscosity. Then, in a manner completely analogous to that detailed in all previously disclosed aspects of the present invention, the blood may be tested for a variety of pathogens. The blood may be accepted or rejected based on the test, which can help prevent the spread of previously heretofore undetectable and/or practically untestable blood borne pathogens.

[086] Of course it is possible to imagine a very large number of permutations of the various aspects of the disclosed invention, however it should be clear that these permutations as will occur to those skilled in the art are within the scope of the invention.

What is claimed is:

1. An apparatus for molecular processing, comprising:
a receptacle defining an interior space and having at least one interior surface bearing a plurality of molecular imprints thereon, the molecular imprints capable of adsorbing at least one selected molecule;
an inlet for selectively introducing a sample flow into the receptacle; and,
an outlet for selectively releasing the sample flow from the receptacle.
2. The apparatus of claim 1, further comprising means for desorbing molecules adsorbed by the molecular imprints.
3. The apparatus of claim 2, wherein the means for desorbing comprises means for heating at least one of the surface bearing the molecular imprints and the sample.
4. The apparatus of claim 2, wherein the means for desorbing comprises a transducer for directing ultrasonic energy onto the surface bearing the molecular imprints.
5. The apparatus of claim 1, wherein the interior surface comprises at least one sensor capable of detecting at least one selected molecule.
6. The apparatus of claim 5, wherein the sensor is a mass sensitive sensor having at least one imprint of the selected molecule on an active surface.
7. The apparatus of claim 5, further comprising means for interrogating the sensor.
8. The apparatus of claim 7, wherein the means for interrogating the sensor is a wireless interrogator.
9. The apparatus of claim 7, wherein the means for interrogating the sensor measures a frequency shift of the sensor.
10. The apparatus of claim 5, further comprising means for calibrating the sensor.

11. The apparatus of claim 10, wherein said means for calibrating the sensor is incorporated in the sensor.
12. The apparatus of claim 1, further comprising means for applying one of an electric and a magnetic field to the surface bearing molecular imprints and the sample flow in the receptacle.
13. The apparatus of claim 1, wherein the at least one interior surface includes imprints of at least two different molecules.
14. The apparatus of claim 13, wherein the imprints of the at least two different molecules on the interior surface are arranged as at least two separate regions.
15. The apparatus of claim 14, wherein the imprints of the at least two different molecules on the interior surface are arranged adjacently.
16. The apparatus of claim 1, further comprising at least one reservoir connected to the vessel, each at last one reservoir for introducing a selected fluid to the receptacle.
17. The apparatus of claim 1, further comprising an additional vessel connected to receive an outflow from the vessel, the additional vessel having an imprinted surface in an interior thereof and having an inlet to receive at least one selected fluid for further processing the outflow.
18. The apparatus of claim 1, further comprising a reservoir connected to receive a flow from the outlet of the receptacle, the reservoir including at least one of a filter and biological deactivation device.
19. The apparatus of claim 1, wherein the surface bearing the plurality of imprints is formed on a substrate removably positioned in the receptacle.
20. The apparatus of claim 19, wherein the substrate further comprises an identification device.
21. The apparatus of claim 19, wherein the substrate further comprises a sensor capable of

detecting at least one selected molecule.

22. The apparatus of claim 21, further comprising means for calibrating the sensor.

23. The apparatus of claim 1, further comprising means for determining a concentration of the sample flow in the receptacle, wherein concentration is used to control an additional flow into the receptacle.

24. The apparatus of claim 1, further comprising electrodes disposed in spaced relationship on the interior surface, means for energizing the electrodes, a radio frequency wave generator to direct RF energy onto the interior surface, and means for measuring an impedance change, wherein, absorption of analyte onto the interior surface is measured.

25. The apparatus of claim 1, further comprising means for directing light onto the interior surface and means for measuring an adsorbance spectrum of reflected light therefrom, wherein, absorption of analyte onto the surface is measured.

26. A method of processing molecular level materials, comprising the steps of:
 adsorbing from a sample selected molecules on a surface bearing at least a plurality of imprints of the selected molecules;
 removing unwanted substances from the sample; and,
 introducing components for reaction with the selected molecules.

27. The method of claim 26, further comprising the step of desorbing the adsorbed molecules.

28. The method of claim 27, wherein the adsorbed molecules are desorbed by heating the surface.

29. The method of claim 26, wherein the components are allowed to react with the adsorbed molecules.

30. The method of claim 26, further comprising the step of desorbing the reacted molecules into a flow.

molecules to facilitate reaction with the introduced components.

32. The method of claim 26, wherein different molecules are adsorbed on the sheet.

33. The method of claim 32, further comprising the step of simultaneously desorbing different molecules into a solution.

34. The method of claim 33, wherein the desorbed different molecules are reacted.

³⁵
~~34~~. The method of claim 26, further comprising sensing at least one of a rate and a quantity of molecules adsorbed on the surface.

³⁶
~~35~~. The method of claim 26, wherein adsorbing molecules includes adsorbing molecules on a surface of a sensor for monitoring the process for the presence of the at least one selected molecule.

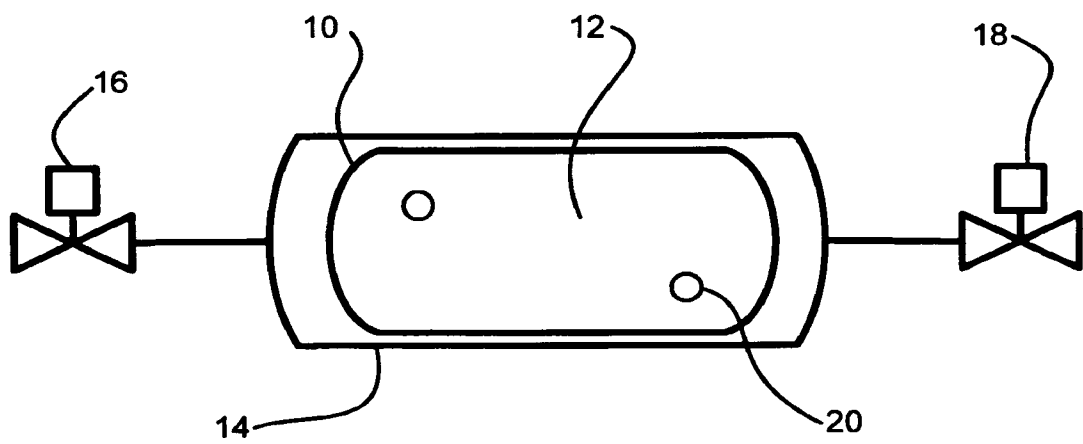


Figure 1

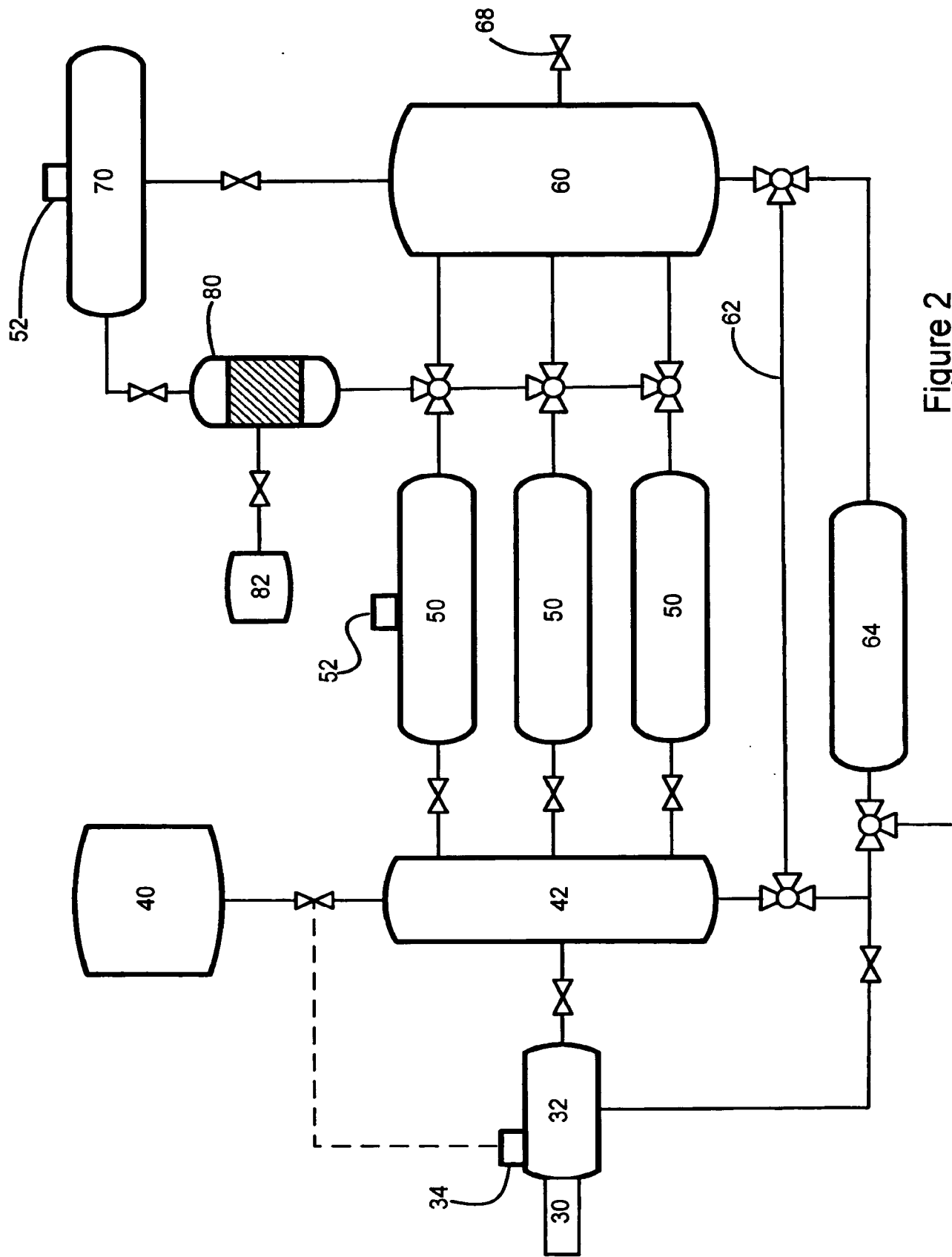


Figure 2