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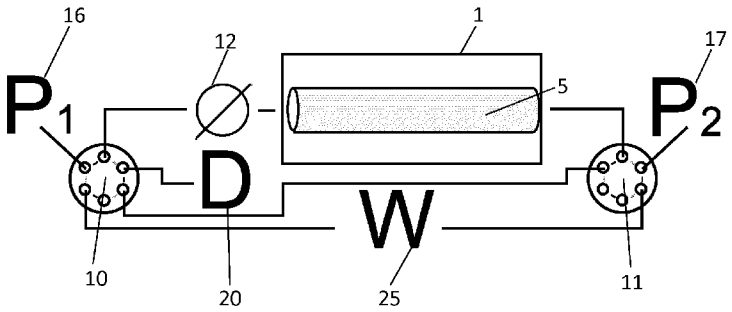


Fig. 2

(57) Abstract: The present subject matter relates to a device for trapping and eluting a liquid chromatography eluate. The device comprises a thermal modulator configured such that it may be fitted with a trapping column located at least substantially within the thermal modulator, and a temperature control unit operationally coupled to the thermal modulator. The thermal modulator has a heating, a cooling and a thermal maintenance mode. In the heating mode the thermal modulator is configured to increase the temperature of the trapping column when fitted by at least 50 °C at a rate of at least 1 °C per second; in the cooling mode the thermal modulator is configured to decrease the temperature of the trapping column when fitted by at least 50 °C at a rate of at least 1 °C per second; and in thermal maintenance mode the thermal modulator is configured to maintain the temperature of the trapping column when fitted. The device may be used in a 2D-LC system. The device may be used in a method of on-line solvent switching for an effluent from a liquid chromatography column. The device may focus analytes

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Liquid chromatography device

[0001] This invention relates to a device that provides solvent switching and/or focusing for one or more analytes in liquid chromatography, for example in two-dimensional liquid chromatography. The invention also relates to methods that use the device to transfer analytes from one solvent to another and/or focus one or more analytes separated by liquid chromatography.

BACKGROUND

[0002] Chromatography is a method used to separate mixtures into components. It uses the differences in partitioning behavior of analytes between a mobile phase and a stationary phase to separate components, which can then be detected. Components of a mixture may interact with the stationary phase depending on their physico-chemical properties, such as charge, polarity relative solubility, adsorption, etc. Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid, with the sample being forced through a column that is typically packed with particulate materials or filled with a porous monolith skeleton (the stationary phase) by a liquid (mobile phase) at a specified pressure.

[0003] For separation of complex mixtures it is often helpful to use two dimensions of chromatographic separation, i.e. two different separation stages. This permits the use of different physico-chemical properties for separation in each of the separation stages, improving the selectivity and peak capacity of the overall separation. Comprehensive two-dimensional liquid chromatography (LC×LC) is the separation approach of choice for detailed analysis of complex, non-volatile mixtures. This is because LC×LC provides much higher peak capacities, that is separation power, than one-dimensional LC.

[0004] A standard way to perform LC×LC involves a first-dimension separation that is relatively slow, e.g. with a typical analysis time of 1 h or longer. Fractions of the effluent from this first-dimension column are collected in a loop of a switching valve. When this valve is switched, the fraction is injected onto a second column, which provides a much faster separation, typically with an analysis time of three minutes or less. While one fraction is being analysed on the second-dimension column, a new fraction is being collected in another loop connected to the same valve (or by using a second valve).

[0005] Thus a standard interface between the first and second separation dimensions consists of a switching valve equipped with two collection loops. Both dimensions are run continuously as the two loops are alternately switched between collecting a first dimension fraction and injecting it into the second dimension. This interface system provides

modulation between the first and second separation dimensions.

[0006] Operational drawbacks of this fraction collection and injection interface often hamper full realization of the method's potential resolving power. In particular, there are a number of factors considered to contribute to modulation induced decrease in efficiency of LC×LC analyses including: asymmetry of collection loops (A. Van der Horst, P.J. Schoenmakers, Journal of Chromatography A 1000 (2003) 693), lengthy modulation cycles (John V Seeley, Journal of Chromatography A, 962 (2002) 21–27), viscosity mismatch between the mobile phases of the first and second dimension (B.S. Broyles, R.A. Shalliker, D.E. Cherrak, G. Guiochon,Journal of Chromatography A 822 (1998) 173), solvent immiscibility (P. Dugo, O. Favoino, R. Luppino, G. Dugo, L. Mondello, Analytical Chemistry, 76 (2004) 2525-2530), the 1D eluent being too strong an injection solvent in the 2D, (X. Jiang, A. van der Horst, P.J. Schoenmakers, Journal of Chromatography A 982 (2002) 55), 1D eluent being too weak an injection solvent in 2D, (E. Reingruber, J.J. Jansen, W. Buchberger, P. Schoenmakers, Journal of Chromatography A, 1218 (2008) 1147-52), and relatively large injection volumes (G. Vivó-Truyols, Si. Van der Wal and P.J. Schoenmakers. Analytical Chemistry 82 (2010) 8525-8536).

[0007] Modulation in comprehensive two-dimensional gas chromatography (GC×GC) has been studied in depth and is routinely employed in a universal and robust way. In GC×GC the modulation principle is usually based on effects of temperature upon retention. This is illustrated in US 7,284,409 B1. The eluting compounds from the first dimension are trapped by rapid, local, cooling and re-injected into the second dimension by heating. This reflects the general approach taken in GC, where an increase in temperature (e.g. in a temperature gradient) is used to promote elution of analytes. Unlike LC×LC, GC×GC mainly involves differences in the stationary phases, and typically both the GC dimensions use the same carrier gas. Therefore the modulation methods used in GC×GC are not directly applicable to LC×LC, where a change of mobile phase is desirable.

[0008] LC×LC and other forms of 2D-LC commonly use diluting make-up flows between separation dimensions at the expense of complexity and sensitivity. A typical modification interface in LC×LC is a collection loop between the LC dimensions. In some set-ups the modulation interface in LC×LC contains a trapping column, for example in the form of a packed loop, between the dimensions to trap the analytes, focus them and change solvents. The use of this packed-loop modulation interface requires time consuming optimization and significant expertise. In addition, such a modulation interface requires long modulation times, unless it is highly miniaturized, which is difficult.

[0009] There is therefore a need to develop improved modulation devices and methods of modulation for use in LC×LC and other types of 2D-LC.

BRIEF SUMMARY OF THE DISCLOSURE

[0010] In accordance with the present invention there is provided a device that provides focusing and/or solvent switching for analytes in liquid chromatography. The device may be used as a modulation device in two-dimensional liquid chromatography. The device may also be used between chromatographic separation and a detector. The invention also relates to methods that use the device to focus the analyte(s) and / or switch the solvent in which the analyte(s) are dispersed.

[0011] In a first aspect the invention provides a device for trapping and eluting a liquid chromatography eluate. The device comprises a thermal modulator having a heating mode, cooling mode and thermal maintenance mode; a trapping column located at least substantially within the thermal modulator, the trapping column connected to a liquid input and a liquid output that are not located within the thermal modulator; a temperature control unit operationally coupled to the thermal modulator such that the temperature control unit applies the heating mode, cooling mode and thermal maintenance mode. In the heating mode the thermal modulator is configured to increase the temperature of the trapping column by at least 25°C at a rate of at least 1°C per second. In the cooling mode the thermal modulator is configured to decrease the temperature of the trapping column by at least 25°C at a rate of at least 1°C per second. In thermal maintenance mode the thermal modulator is configured to maintain the temperature of the trapping column.

[0012] The thermal modulator may comprise a heater that is activated during the heating mode. The heater may comprise a resistive heater. The thermal modulator may comprise a cooler that is activated during the cooling mode. The cooler may comprise at least one cryogenic jet, where the cryogenic jet is a cryogenic liquid jet or a cryogenic gas jet. For example, the cooler may comprise at least two cryogenic jets, at least three cryogenic jets, or at least four cryogenic jets; e.g. 2, 3, 4, 5 or 6 cryogenic jets. The thermal modulator may comprise the heater and the cooler. The thermal modulator may comprise a chamber. The chamber may comprise the heater and / or the cooler. The chamber may be insulated.

[0013] The thermal modulator may comprise a chamber comprising a bath liquid in thermal communication with the trapping column, such that in the heating mode a hot bath liquid is introduced into the chamber, and in cooling mode a cold bath liquid is introduced into the chamber. The chamber may comprise an inlet and an outlet for the bath liquid, wherein the inlet comprises a valve to switch between the hot bath liquid in heating mode and the cold bath liquid in cooling mode. The chamber may be a heater when the hot bath liquid is introduced. The chamber may be a cooler when the cold bath liquid is introduced. The chamber may be insulated.

[0014] In the heating mode the thermal modulator may be configured to increase the temperature of the trapping column by at least 50°C, optionally by at least 75°C, further optionally by at least 100°C, still further optionally by at least 125°C. For example in the heating mode the thermal modulator may be configured to increase the temperature of the trapping column by at least 50°C, 75°C, 100°C, 125°C, 150°C, 175°C, 200°C, 275°C, or 300°C. In the heating mode the thermal modulator may be configured to increase the temperature of the trapping column at a rate of at least 1°C per second for example at a rate of at least 1.5°C per second, optionally at a rate of at least 2°C per second, further optionally at a rate of at least 2.5°C per second, still further optionally at a rate of at least 3°C per second. For example in the heating mode the thermal modulator may be configured to increase the temperature of the trapping column at a rate of at least 1.5°C per second, at least 2°C per second, at least 2.5°C per second, at least 3°C per second, at least 3.5°C per second, at least 4°C per second, at least 4.5°C per second, at least 5°C per second, at least 6°C per second, at least 7°C per second, at least 8°C per second, at least 9°C per second, or at least 10°C per second. For example in the heating mode the thermal modulator may be configured to increase the temperature of the trapping column at a rate of at least 1.5°C per second, at least 2°C per second, at least 2.5°C per second, at least 3°C per second, at least 3.5°C per second, at least 4°C per second, at least 4.5°C per second, or at least 5°C per second; e.g. at least 3, 4 or 5 °C per second. For example in the heating mode the thermal modulator may be configured to increase the temperature of the trapping column at a rate of at least 5°C per second, at least 6°C per second, at least 7°C per second, at least 8°C per second, at least 9°C per second, or at least 10°C per second. In the heating mode the thermal modulator may be configured to increase the temperature of the trapping column to up to at least100°C, optionally up to at least 150°C, further optionally up to at least 200°C, still further optionally up to at least 250°C, even further optionally up to at least 300°C. For example, in the heating mode the thermal modulator may be configured to increase the temperature of the trapping column to up to at least 200°C, optionally to up to at least 300°C.

[0015] In the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column by at least 50°C, optionally by at least 75°C, further optionally by at least 100°C, still further optionally by at least 125°C. For example in the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column by at least 50°C, 75°C, 100°C, 125°C, 150°C, 175°C, 200°C, 275°C, or 300°C. In the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column at a rate of at least 1°C per second for example at a rate of at least 1.5°C per second, optionally at a rate of at least 2°C per second, further optionally at a rate of at least 2.5°C per second, still further optionally at a rate of at least

3℃ per second. For example in the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column at a rate of at least 1.5℃ per second, at least 2℃ per second, at least 2.5℃ per second, at least 3℃ per second, at least 3.5℃ per second, at least 4℃ per second, at least 4.5℃ per second, at least 5℃ per second, at least 6℃ per second, at least 7℃ per second, at least 8℃ per second, at least 9℃ per second, or at least 10℃ per second. For example in the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column at a rate of at least 1℃ per second, for example at a rate of at least 1.5℃ per second, at least 2℃ per second, at least 2.5℃ per second, at least 3℃ per second, at least 3.5℃ per second, at least 4℃ per second, at least 4.5℃ per second, or at least 5℃ per second; e.g. at least 3, 4 or 5 ℃ per second. For example in the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column at a rate of at least 5℃ per second, at least 6℃ per second, at least 7℃ per second, at least 8℃ per second, at least 9℃ per second, or at least 10℃ per second. In the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column to a temperature of 30℃ or below, e.g. to below 30℃, 20℃, 10℃, 0℃, -10℃, -20℃, -30℃, -40℃, -50℃, -100℃, or -150℃. In the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column to below ambient temperature, e.g. to below 20℃, 10℃, 0℃, -10℃, -20℃, -30℃, -40℃, -50℃, -100℃, or -150℃. In the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column to not more than 20℃, optionally to not more than 10℃, further optionally to not more than 0℃, still further optionally to not more than -10℃, even further optionally not more than -20℃ or -30℃. In the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column to not more than 0℃, optionally to not more than -20℃, further optionally to not more than -50℃, for example to not more than -100℃.

[0016] In the thermal maintenance mode the thermal modulator may be configured to maintain the temperature of the thermal modulator to within a temperature range of ±10℃, ±5℃, ±2℃, ±1℃ or ±0.5℃. For example, in the thermal maintenance mode the thermal modulator may be configured to maintain the temperature of the thermal modulator to within a temperature range of ±2℃, ±1℃ or ±0.5℃, e.g. within a temperature range of ±2℃ or ±1℃. In the thermal maintenance mode the temperature control unit may maintain the temperature of the thermal modulator by selective activation of the heater and / or the cooler. The selective activation may be in response to temperature information provided to the temperature control unit by a temperature monitor that is in thermal contact with the thermal modulator.

[0017] The trapping column may be a preparative chromatography column, high-performance liquid chromatography column, micro LC column, capillary LC column, a nano LC column or a microfluidic device. In an embodiment the trapping column is a capillary LC column, e.g. a monolithic capillary LC column. The support material of the trapping column may be monolithic or packed (particulate), e.g. the support material may be monolithic. The trapping column may comprise a stationary phase selected from reverse phase, normal phase, partition, hydrophilic interaction liquid chromatography (HILIC), ion exchange, affinity and chiral. The trapping column may comprise a reversed phase stationary phase, e.g. a reversed phase monolithic LC column, such as a reversed phase monolithic capillary LC column. In an embodiment the trapping column is not a size-exclusion chromatography (SEC) column. The trapping column may comprise a stationary phase that is resistant to decomposition under high pressure at elevated temperatures (e.g. 150°C, 200°C, 250°C, or 300°C). Stationary phases that are resistant to decomposition at elevated temperature under high pressure may be selected from porous graphitic carbon, metal-oxide, organic polymers and hybrid organic silica, silica phases and other suitable materials.

[0018] In a second aspect the invention provides a device for trapping and eluting a liquid chromatography eluate. The device comprises a thermal modulator having a heating mode, cooling mode and thermal maintenance mode; and a temperature control unit operationally coupled to the thermal modulator such that the temperature control unit applies the heating mode, cooling mode and thermal maintenance mode. The thermal modulator is configured to receive a trapping column located at least substantially within the thermal modulator, the trapping column configured for connection to a liquid input and a liquid output that are not located within the thermal modulator. In the heating mode the thermal modulator is configured to increase the temperature of the trapping column, when fitted, by at least 25°C at a rate of at least 1°C per second. In the cooling mode the thermal modulator is configured to decrease the temperature of the trapping column, when fitted, by at least 25°C at a rate of at least 1°C per second. In thermal maintenance mode the thermal modulator is configured to maintain the temperature of the trapping column when fitted. The device of the second aspect may be as further defined above in relation to the first aspect.

[0019] In a third aspect the invention provides a two-dimensional liquid chromatography (2D-LC) system, comprising a first LC column, a second LC column and a device of the first aspect or second aspect comprising a trapping column, wherein the trapping column is located in fluid connection between the first LC column and second LC column.

[0020] In an embodiment the trapping column has a first end that may act as an inlet or

outlet depending on the operating mode. In a load mode the first end of the trapping column comprises an inlet in fluid connection with an outlet of the first LC column, and in a backflush elute mode the first end comprises an outlet in fluid connection with an inlet of the second LC column.

[0021] In an embodiment the 2D-LC system is a comprehensive 2D-LC system (LC ×LC system). In an embodiment the 2D-LC system is an heart-cutting 2D-LC system, (LC – LC system).

[0022] A fourth aspect provides a method to switch solvents of analytes on-line for an effluent from a liquid chromatography (LC) column. The method comprises (a) providing a device of the first aspect or second aspect comprising a trapping column; (b) setting the thermal modulator to a first thermal maintenance mode, wherein the temperature of the trapping column is maintained at a hot temperature; (c) introducing the effluent from a liquid chromatography column to the liquid input of the trapping column, wherein the effluent comprises a solvent and at least one analyte; (d) setting the thermal modulator to the cooling mode until a cold temperature is reached and then setting the thermal modulator to a second thermal maintenance mode, wherein the temperature of the trapping column is maintained at the cold temperature; (e) stopping introducing the effluent at about the same time as (e.g. within 0.5 s or 1 s), shortly before, or shortly after (e.g. within ±2, ±5, ±10 or ±15 s) setting the thermal modulator to the cooling mode; and (f) introducing an elution solvent to the liquid input or liquid output of the trapping column, such that the at least one analyte is eluted by the elution solvent, the eluted at least one analyte and elution solvent forming a modulated effluent. This will provide one or more advantages. For example, the at least one analyte will be transferred from the effluent solvent to the elution solvent. The elution solvent may be selected for better compatibility with the method of detection and / or further dimension of chromatography.

[0023] The elution solvent may have a higher elution strength than the solvent of the effluent. Where the elution solvent has a higher elution strength than the solvent, the at least one analyte may be focussed, which would be expected to provide a number of advantages in further analysis and detection, e.g. providing one or more of more increased separation efficiency, a higher signal and/or better signal to noise for detection.

[0024] Without wishing to be bound by any theory, in the method of the invention the trapping column may be loaded until the least retained analyte is about to elute, without significant loss of any analyte. The amount of effluent introduced may be up to about three, two or one volume of the trapping column. As the skilled person will appreciate, it may also be possible to introduce more than three trapping column volumes without significant loss of analyte (e.g. up to 5, up to 10, up to 20 or up to 50 column volumes),

dependent on the volume required to elute the analyte. The amount of effluent introduced may be about one volume of the trapping column. About one volume may be from about 0.8 to about 1.2 column volume, e.g. from about 0.9 to about 1.2 column volume. Introducing effluent up to about one volume of the trapping column avoids loss of any analyte that may be minimally retained on the trapping column at the hot temperature.

[0025] Step (e) may be performed at about the same time as step (d), for example steps (d) and (e) may be performed within 0.01, 0.1, 0.25, 0.5 or 1 s of one another. Step (e) may be performed after step (d), for example stopping introducing the effluent may occur not more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 s after setting the thermal modulator to the cooling mode, e.g. not more than 5, optionally 10, further optionally 15 s after setting the thermal modulator to the cooling mode. Step (e) may be performed before step (d), for example stopping introducing the effluent may occur not more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 s before setting the thermal modulator to the cooling mode, e.g. not more than 5, optionally not more than 10, further optionally not more than 15 s before setting the thermal modulator to the cooling mode.

[0026] Step (f) may further comprise introducing the modulated effluent to a second liquid chromatography column, or a detector, or collecting the modulated effluent.

[0027] The method may further comprise (g) setting the thermal modulator to the heating mode until the hot temperature is reached, and then repeating steps (b) – (f). The method may further comprise repeating steps (b) – (g) one or more times, for example 2, 3, 4, 5, 6, 7, 8, 9 or 10 times; or about 50, about 100, about 150 or about 200 times. The method may comprise repeating steps (b) – (g) up to 200 times or up to 400 times, e.g. when the method is a 2D – LC method (e.g. an LC×LC method) and step (f) further comprises introducing the modulated effluent to a second liquid chromatography column. When steps (a) – (f) are repeated, each repetition may relate to an individual fraction of effluent from the LC column.

[0028] The method may be a back flush method, wherein the elution solvent is introduced into the liquid output of the trapping column. In a back flush method, the trapping column is flushed with the elution solvent in the opposite direction to the flow of the effluent from the LC column. The back flush method may provide better focussing for the at least one analyte compared to conventional (i.e. forward flush) methods.

[0029] The hot temperature may be at least 25°C hotter than the cold temperature. The hot temperature may be at least 50°C hotter than the cold temperature, optionally at least 100°C hotter than the cold temperature, further optionally at least 200°C hotter than the cold temperature, still further optionally at least 250°C hotter than the cold temperature. For example, the hot temperature may be at least 50°C, 75°C, 100°C, 125°C, 150°C,

175℃, 200℃, 225℃ or 250℃ hotter than the cold temperature.

[0030] In the cooling mode the thermal modulator may decrease the temperature of the trapping column by at least 25℃. In the cooling mode the thermal modulator may decrease the temperature of the trapping column by at least 50℃, optionally by at least 75℃, further optionally by at least 100℃, still further optionally by at least 125℃. For example in the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column by at least 50℃, 75℃, 100℃, 125℃, 150℃, 175℃, 200℃, 275℃, or 300℃. In the cooling mode the thermal modulator may decrease the temperature of the trapping column at a rate of at least 1.5℃ per second, optionally at a rate of at least 2℃ per second, further optionally at a rate of at least 2.5℃ per second, further optionally at a rate of at least 3℃ per second. In the heating mode the thermal modulator may increase the temperature of the trapping column to up to at least 200℃, optionally to up to at least 300℃. For example in the cooling mode the thermal modulator may decrease the temperature of the trapping column at a rate of at least 1.5℃ per second, at least 2℃ per second, at least 2.5℃ per second, at least 3℃ per second, at least 3.5℃ per second, at least 4℃ per second, at least 4.5℃ per second, at least 5℃ per second, at least 6℃ per second, at least 7℃ per second, at least 8℃ per second, at least 9℃ per second, or at least 10℃ per second. For example in the cooling mode the thermal modulator may decrease the temperature of the trapping column at a rate of at least 1.5℃ per second, at least 2℃ per second, at least 2.5℃ per second, at least 3℃ per second, at least 3.5℃ per second, at least 4℃ per second, at least 4.5℃ per second, or at least 5℃ per second; e.g. at least 3, 4 or 5 ℃ per second. For example in the cooling mode the thermal modulator may be configured to decrease the temperature of the trapping column at a rate of at least 5℃ per second, at least 6℃ per second, at least 7℃ per second, at least 8℃ per second, at least 9℃ per second, or at least 10℃ per second. In the cooling mode the thermal modulator may decrease the temperature of the trapping column to a temperature of 30℃ or below, e.g. to below 30℃, 20℃, 10℃, 0℃, -10℃, -20℃, -30℃, -40℃, -50℃, -100℃, or -150℃. In the cooling mode the thermal modulator may decrease the temperature of the trapping column to below ambient temperature, e.g. to below 20℃, 10℃, 0℃, -10℃, -20℃, -30℃, -40℃, -50℃, -100℃, or -150℃. In the cooling mode the thermal modulator may decrease the temperature of the trapping column to not more than 20℃, optionally to not more than 10℃, further optionally to not more than 0℃, still further optionally to not more than -10℃, even further optionally not more than -20℃ or -30℃ For example to below 0℃, -10℃, -20℃, -30℃, -40℃, -50℃, -100℃, or -150℃. In the cooling mode the thermal modulator may decrease the temperature of the trapping column to not more than 0℃, optionally to not more than -20℃, further optionally to not more than -50℃, for example to not more than -100℃.

[0031] The hot temperature may be at least 100°C, optionally at least 200°C, further optionally at least 300°C. The cold temperature may be not more than 30°C, 20°C, 10°C, 0°C, -10°C, -20°C, -30°C, -40°C, -50°C, -100°C, or -150°C; e.g. not more than 10°C, 0°C, -10°C, -20°C, -30°C, -40°C, -50°C or -100°C. The cold temperature may be not more than 0°C, optionally to not more than -20°C, further optionally not more than -50°C, still further optionally not more than -100°C.

[0032] In a fifth aspect the invention provides use of the device of the first or second aspect as a modulator between the first and second liquid chromatography dimensions in a two-dimensional liquid chromatography (2D-LC) system. In an embodiment the 2D-LC system is a comprehensive 2D-LC system (LC × LC system). In an embodiment the 2D-LC system is an heart-cutting 2D-LC system (LC – LC system). In an embodiment the 2D-LC system is a selective comprehensive two-dimensional liquid chromatography system (s LC × LC system).

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which:

Figure 1 is a diagrammatic representation of four different loading and elution schemes.

Figure 2 illustrates a device of the invention comprising a thermal modulator and a trapping column configured as part of a LC system.

Figure 3a to 3c illustrates a device of the invention comprising a thermal modulator and a trapping column configured as part of a 2D - LC system.

Figure 4 illustrates a device of the invention where the thermal modulator comprises a heater that comprises a resistive heater and a cooler that comprises cryogenic jets.

Figure 5 illustrates a device of the invention where the thermal modulator comprises a chamber adapted to be filled with a bath liquid.

Figure 6 illustrates the retention of the analytes toluene, 1,4-dimethylbenzene and benzophenone on a butyl methacrylate-ethylene dimethacrylate (BUMA-EDMA) monolithic column at 0°C and 150°C.

Figure 7 illustrates how loading a trapping column while hot and eluting while cold (hot in – cold out) provides solvent switching.

Figure 8 illustrates the focussing effect of using a solvent with a higher elution strength than the loading solvent for elution in a hot in-cold out method.

DETAILED DESCRIPTION

[0034] Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of them mean "including but not limited to", and they are not intended to (and do not) exclude other moieties, additives, components, integers or steps. Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

[0035] Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith. All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. The invention is not restricted to the details of any foregoing embodiments. The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

[0036] The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

[0037] For the avoidance of doubt, it is hereby stated that the information disclosed earlier in this specification under the heading "Background" is relevant to the invention and is to be read as part of the disclosure of the invention.

DEFINITIONS

[0038] The following explanations of terms and methods are provided to better describe the present disclosure and to guide those of ordinary skill in the art in the practice of the present disclosure.

[0039] "Liquid chromatography" (LC) is a separation technique used to separate components of a sample. The sample containing analyte is dissolved in a solvent (if the sample is not a liquid sample) and introduced into a column comprising a stationary phase.

A liquid mobile phase is forced through the column and differences in the partitioning of analytes between the stationary phase and mobile phase results in separation of the analytes in the effluent of the column. The active part of the stationary phase is typically present on a support, e.g. a particulate support for a packed column or a monolithic support for a monolithic column. Exemplary scales of LC, which differ on the basis of the size of the columns used include preparative chromatography (column diameter typically at least 4.6 mm), high-performance liquid chromatography (HPLC) (column diameters typically 4.6 – 2 mm), micro LC (column diameter typically 1 – 0.5 mm), capillary LC (column diameter typically 0.5 – 0.1 mm) and nano LC (column diameter typically 0.1 – 0.05 mm). Exemplary forms of liquid chromatography that differ on the basis of the type of stationary phase / mobile phase / analyte interaction used include reverse phase LC, normal phase LC, partition LC, ion exchange LC, affinity LC and chiral LC. It will be appreciated that any exemplary scale of LC may be used with any exemplary form of LC.

[0040] The term "eluent" refers to the carrier portion of the mobile phase that is introduced to an LC.

[0041] The terms "eluate" or "effluent", which may be used interchangeable herein, refer to the liquid that emerges from an LC, comprising the mobile phase and, when present, analyte(s), e.g. the mobile phase and at least one analyte.

[0042] The term "modulator" refers to a device that is adapted for use at the output of an LC column to alter the relationship between the eluent and solute (e.g. analyte material) of the eluate. A modulator of (or of use in) the invention may provide solvent switching. Solvent switching comprises replacing the eluent with a different solvent system. A modulator of (or of use in) the invention may provide focusing. Focusing comprises concentrating the analyte into a smaller volume of stationary phase and/or solvent. A modulator of (or of use in) the invention may provide solvent switching and / or focusing. A modulator of (or of use in) the invention may be used as an interface between different dimensions of LC separation, e.g. between the first and second LC dimensions in 2D – LC (e.g. in LC×LC). A modulator may also be used as an interface between an LC system and an LC detector.

[0043] The term "rate" when used in relation to a rate of heating or cooling, refers to how quickly the temperature increases (heating) or decreases (cooling), typically specified in degree centigrade (℃) per second. The rate may refer to an average rate over a period of time. This period of time may be at least 5 s or at least 10 s. This period of time may be at least 10 s, at least 20 s, at least 30 s, at least 40 s, at least 50 s, or at least 60 s. The period of time may be not more than 10 s, not more than 20 s, not more than 30 s, not more than 40 s, not more than 50 s, or not more than 60 s. For example the period of time

may be between about 10 s and about 60 s or between about 10 s and about 30 s or be between about 5 s and about 30 s, e.g. between about 5 s and about 20 s. The period of time may be about 5 s, about 10 s, about 20 s, about 30 s, about 40 s, about 50 s, or about 60 s. Having a relatively fast rate of heating or cooling may be advantageous, for example for comprehensive 2D-LC, as this may assist in more efficient real time analysis.

[0044] In some embodiments, for example heart-cutting 2D - LC, having a fast rate of heating or cooling may be less critical. For such embodiments, the period of time (in relation to a rate of heating or cooling) may be at least 1 minute, at least 2 minutes, at least 3 minutes, at least 4 minutes, at least 5 minutes, at least 6 minutes, at least 7 minutes, at least 8 minutes, at least 9 minutes or at least 10 minutes. The period of time may be not more than 1 minute, not more than 2 minutes, not more than 3 minutes, not more than 4 minutes, not more than 5 minutes, not more than 6 minutes, not more than 7 minutes, not more than 8 minutes, not more than 9 minutes or not more than 10 minutes. For example the period of time may be between 1 minute and 5 minutes or between 1 minute and 10 minutes. The period of time may be about 1 minute, about 2 minutes, about 3 minutes, about 4 minutes, about 5 minutes, about 6 minutes, about 7 minutes, about 8 minutes, about 9 minutes or about 10 minutes.

[0045] The term "two-dimensional liquid chromatograph" (2D – LC) refers to a form of LC that uses a first LC dimension with a column that uses a first stationary phase and a second LC dimension that uses a second stationary phase. The second stationary phase is typically different to the first stationary phase. The first and second LC dimensions are typically connected with an interface system that provides modulation between the first and second separation dimensions. At least four typical on-line modes of 2D – LC may be used. These modes include comprehensive 2D – LC (LC × LC), heart-cutting 2D - LC is used (LC – LC), selective comprehensive two-dimensional Liquid Chromatography (sLC × LC) and stop flow 2D – LC. In LC × LC the entire sample is subject to two separation dimensions, i.e. the sample is loaded onto the first LC dimension and all parts (e.g. fractions) of the first LC dimension's chromatogram are subjected to the second LC dimension. In LC – LC selected parts (e.g. fractions) of the first LC dimension's chromatogram are subjected to the second LC dimension separation. The sLC × LC mode involves comprehensive operation (i.e. analysing all fractions) within one or more specified range(s) of the first-dimension chromatogram. This may, for example, be achieved by parking first dimension segments in multiple loops and eventually analysing them in the second dimension. These might be run continuously or by stopping flow in the first dimension to allow for longer second dimension run times (stop flow 2D – LC).

APPARATUS AND METHODS

[0046] Figure 1 illustrates four different schemes for modulation in LC, labeled (i), (ii), (iii) and (iv). In all four schemes the modulator comprises a trapping column, represented by a cylinder, onto which an effluent comprising three different analytes, represented by a square, circle and triangle, are loaded. The direction of the flow during loading is illustrated by an arrow in the topmost illustration (step a) in each of the schemes. In all four schemes the analytes are backflush eluted from the trapping column, i.e. the elution solvent is pumped through the column in the opposite direction to the loading effluent. This is illustrated by the direction of the arrow in illustration (e) in each of the schemes.

[0047] Scheme (i) is a representation of a traditional trapping method where the trapping step is performed on a cold column (steps a – c) increasing retention and the capacity of the column. One column volume may be loaded and the analyte is retained near the head of the column. The trap is then heated up to decrease retention (step d) and the analytes are backflush eluted (steps e – g). This elutes the analytes quickly, achieving a limited amount of focusing, but does not provide solvent switching.

[0048] Scheme (ii) is a representation of a hot-in-hot-out trapping scenario, which is included for comparative purposes. The column is both loaded and eluted while hot. This offers neither focusing of analytes, nor solvent switching.

[0049] Scheme (iii) illustrates a hot-in-cold-out method of the invention. In this illustration the solvent of elution has the same (or similar) elution strength as the loading solvent. The column is loaded at a hot temperature and the analytes have low retention. After about one column volume is loaded (step d), the column is cooled and the analytes are eluted in a backflush manner (steps e – g). The decrease in temperature means that the analytes are better retained on the stationary phase when compared to what occurred during loading. The analytes therefore pass the solvent boundary between the loading and elution solvents, achieving the advantage of solvent switching, but not focusing.

[0050] Scheme (iv) illustrates the hot-in-cold-out method of the invention, further comprising an elution solvent of a higher elution strength than the loading solvent. In this approach steps a – d are as per scheme (iii), i.e. about one column volume is loaded and the column is then cooled. While the column is cool, the elution solvent of higher elution is used to elute the analytes in a backflush manner. The analytes are transferred across the solvent boundary (as in scheme (iii)). The analytes are also concentrated at the elution solvent front due to the strength of the elution solvent this provides the advantage of focusing the analytes. While schemes (iii) and (iv) have been illustrated with a backflush elution, it is also possible to employ similar hot-in-cold-out methods using forwardflush elution, although forwardflush methods may provide reduced focusing compared to the

backflush methods.

[0051] The ability to provide solvent switching (and optionally focusing) means that the methods of the invention are useful in any application where it is desired to change the solvent from that of the LC effluent. For example, in 2D-LC (e.g. LC×LC) it may be advantageous to switch the solvent between the first and second LC dimensions, e.g. where the first LC dimension's effluent comprises a solvent that is not compatible with the second LC dimension. For example, in LC coupled with nuclear-magnetic-resonance spectroscopy (LC-NMR) it may be advantageous to change from the effluent solvent to an isotopically labelled solvent (e.g. switching to deuterated solvent for protonNMR). For example, in LC-MS it may be advantageous to solvent switch to a volatile solvent system, which has better compatibility with the MS ionization technique being used. In addition, in all of these examples (2D-LC, LC-NMR, LC-MS) focusing of the analytes by the methods of the invention provides the advantage that lower detection limits may be reached.

[0052] The hot-in-cold-out methods of modulation provide the advantages of solvent exchange and/or focusing of analytes for many different stationary phases and suitable mobile phases. The reason for this is that most conventional forms of LC show changes in retention with a change in temperature. For example reversed phase, normal phase, ion-exchange, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC) and bio-affinity chromatography are all suitable for use in the present invention. Size-exclusion chromatography SEC) does not show changes in retention behavior of analytes with with changes in temperature, therefore the present invention does not involve methods or devices that use a SEC trapping column.

[0053] The effect of temperature on LC solute retention factor, k, can be expressed by the van't Hoff equation:

$$\log(k) = -\frac{\Delta H_0}{2.3RT} + \frac{\Delta S_0}{2.3R} + \log \Phi \qquad (1)$$

ΔH_0 and ΔS_0 are respectively the molar enthalpy and entropy of solute transfer from the mobile phase to the stationary phase, T is the temperature in Kelvin, R is the universal gas constant and Φ is the phase ratio of the column (the volume of the stationary phase divided by the volume of the mobile phase). When the slope of ($\Delta H_0/2.3R$) equals zero, e.g. as expected for SEC trapping columns and methods, the device is not a device of the invention as changing the temperature does not affect the strength of the interaction between the analyte and the stationary phase.

[0054] Without wishing to be bound by any theory, it is believed that, with the exception of SEC trapping columns, substantially all conventional forms of LC show changes in retention with a change in temperature, i.e. $\Delta H^0/2.3R$ in equation 1 is not equal to zero.

The examples in the present application illustrate the effects of temperature on retention for reversed phase chromatography. That temperature effects on retention are generally applicable in reversed phase chromatography is further supported by S. Heinisch and J.L. Rocca, Journal of Chromatography A 1216 (2009) 642 – 658 (see in particular at section 2.2.1 “Effect of temperature on retention” and figure 7). Temperature effects have also been observed with other forms of LC, for example HIC (see Vailaya, A. and Horváth, C. Ind. Eng. Chem. Res. 35 (1996), 2964 -2981 specifically part 7 Conclusions). Changes in retention of analytes in response to changes in temperature would therefore be expected with reverse phase LC and HIC LC, as well as with other forms of LC.

[0055] Figure 2 provides a schematic illustration of an LC system that comprises a device of the invention. This device comprises a thermal modulator 1 which has a heating mode, cooling mode and thermal maintenance mode and at least substantially encloses a trapping column 5. The LC system further comprises a first valve 10, second valve 11 and injection valve 12. The first valve comprises fluid connections to the second valve 11, injection valve 12, pump P_1 16, output (D) 20 and waste 25. The injection valve 12 also comprises a fluid connection to the trapping column 5 and an injection loop. The injection loop may be filled with a fraction of the effluent from an LC separation by manual injection or by automated injection. The second valve 11 also comprises fluid connections to pump P_2 17 and waste 25. While the valves 10, 11 and 12 and thermal modulator 1 may be manually controlled, typically these components are controlled by one or more computers and programs, permitting automated control of the LC system. For example, the thermal modulator may be operationally coupled to a computerized temperature control unit, which controls the heating mode, cooling mode and thermal maintenance mode, and may optional also comprise a sensor for monitoring the temperature of the trapping column 5.

[0056] The LC system of figure 2 can be used in the following method of the invention. When the first valve 10 is in position A (indicated by the dark lines) pump P_1 16 pumps solvent into the injection valve and loads the trapping column 5 with the content of the injection loop. This loading step is performed while the trapping column is maintained at a hot temperature by the thermal modulator 1. When the first valve 10 is in position B (indicated by the faded lines) the trapping column is brought off-line and pump P_1 16 is diverted to waste. The first valve may be switched from position A to position B, e.g. when about one trapping column volume of solvent has been pumped through the trapping column 5. The thermal modulator 1 is set to the cooling mode and the trapping column 5 is cooled to a cold temperature. The trapping column 5 is maintained at the cold temperature and the second valve 11 is switched from position A (dark lines) where pump P_2 17 is diverted to waste, to position B (faded lines) where pump P_2 17 is in fluid

connection with the trapping column 5. This results in the trapping column 5 being eluted in a backflush manner, with its eluate passing to output (D) 20. As the skilled person will appreciate, output (D) 20 may represent a detector, such as a UV, MS or NMR detector. Output (D) 20 may also be a further dimension of analytical LC separation, e.g. the second dimension of analytical LC separation in a 2D-LC (e.g. LC×LC) system, or an injection loop, the content of which is to be injected into the second dimension of a 2D-LC system. After backflush elution is complete, the second valve 11 may be switched from position B back to position A and the thermal modulator may be set to the heating mode to heat the trapping column 5 to the hot temperature.

[0057] While the first valve 10 is in position B and the second valve 11 is in position A the injection loop of the injection valve may be refilled with an effluent from an LC separation. The method of the above paragraph may then be repeated. As the skilled person will appreciate, repetition of this method allows sequential trapping and elution of fractions from an LC run.

[0058] Figure 3(a) to 3(c) provides a schematic illustration of a 2D-LC system of the invention (e.g. an LC×LC system) that comprises a device for trapping and eluting the analyte(s) from a liquid chromatography effluent of the invention. Similarly to figure 2, the device comprises a thermal modulator 1 which has a heating mode, cooling mode and thermal maintenance mode and at least substantially encloses a trapping column 5 (trapping column not illustrated). The 2D-LC system also comprises a first dimension valve 30, second dimension valve 31 and trapping column valve 32; with a fluid connection 33 between the first dimension valve 30 and the trapping column, and two fluid connections 34, 35 between the second dimension valve 31 and the trapping column. The first dimension valve 30 comprises fluid connections for the first dimension LC effluent 40, first loop 41, second loop 42, modulator loading solvent 43, and waste 45. The second dimension valve comprises fluid connections for the second dimension LC mobile phase 50, the second dimension LC column input 51, waste 45 and modulator elution solvent 52. The valves 30, 31, 32 and thermal modulator 1 may be controlled by one or more computers and programs, permitting automated control of the LC system. One or more of the pumping systems providing the first dimension LC effluent 40, modulator loading solvent 43, second dimension LC mobile phase 50 and modulator elution solvent 52 may be controlled by one or more computers and programs, permitting automated control of the LC system. Such computer control systems are known in the art, and may be adapted for use with the methods and devices of the invention.

[0059] In figure 3(a) each of the first dimension valve 30, the second dimension valve 31 and the trapping column valve 32 are set to their position A. In this configuration the first

dimension LC effluent 40 is collected in the first loop 41 with excess going to waste 45. The content of the second loop 42 (e.g. previously collected first dimension LC effluent 40) is loaded onto the trapping column by the modulator loading solvent 43. The modulator eluting solvent 52 is diverted to waste 45. The second dimension LC mobile phase 50 is connected to the second dimension LC column input 51, to drive the second dimension of LC separation. As the trapping column is being loaded, the trapping column is maintained at a hot temperature by the thermal modulator 1, as described herein.

[0060] In figure 3(b) the trapping column valve 32 has been switched to its position B. Either shortly before, during, or immediately after this switching, the thermal modulator 1 is set to the cooling mode until the trapping column reaches a cold temperature, then the thermal modulator 1 is set to maintain the trapping column at the cold temperature. Switching the trapping column valve 32 to position B diverts the modulator loading solvent 43 to waste 45, but causes the modulator eluting solvent 52 to backflush elute analytes from the trapping column into fluid connection 35 (located between the the trapping column valve 32 and the second dimension valve 31).

[0061] In figure 3(c) the second dimension valve 31 has been switched to its position B. The second dimension LC mobile phase 50 is now diverted through the trapping column and injects the backflush eluted analytes from fluid connection 35 to the second dimension LC column input 51, thereby injecting the analytes onto the second dimension column. After the analytes have been injected onto the second dimension column, the second dimension valve 31 is switched to position A (the valve configuration of figure 3(b)), so that the second dimension LC mobile phase 50 is connected to the second dimension LC column input 51, to drive the second dimension of LC separation for the analytes.

[0062] The thermal modulator 1 may then be set to the heating mode until the trapping column reaches the hot temperature, then the thermal modulator 1 is set to maintain the trapping column at the hot temperature. The trapping column valve 32 may then be switched to its position A and the first dimension valve 30 to its position B. In this configuration the first dimension LC effluent 40 is collected in the second loop 41 with excess going to waste 45. The content of the first loop 41 (previously collected first dimension LC effluent 40) is loaded onto the trapping column by the modulator loading solvent 43. The modulator eluting solvent 52 is diverted to waste 45. The second dimension LC mobile phase 50 is connected to the second dimension LC column input 51, to drive the second dimension of LC separation. As the trapping column is being loaded, the trapping column is maintained at a hot temperature by the thermal modulator 1, as described herein. The valve switching and temperature modulation described above in relation to figures 3(b) and 3(c) is then repeated. This overall process can be repeated

until the 2D-LC experiment has been completed. In a typical LC×LC mode 2D-LC experiment more than ~200 modulations may be done per analysis. In addition, it is beneficial for the solvent switching to be fast, to avoid undersampling of the first dimension of LC separation.

[0063] Figures 4 and 5 illustrate in schematic form devices that may be used as the thermal modulator and trapping column in an embodiment of the invention (e.g. as illustrated in figure 2 and figure 3(a) to 3(c)). In figure 4 the device comprises a thermal modulator 1 and a trapping column 5. The thermal modulator 1 comprises a cooler comprising cryogenic cooling jets 100 that provide a cryogen 101. The cryogen may be a cryogenic liquid, e.g. liquid nitrogen. The cryogen may be a chilled gas, e.g. carbon dioxide or nitrogen. While the cooler is illustrated with 5 cryogenic cooling jets 100, it will be appreciated that the device may contain a larger or smaller number of cryogenic jets 100, i.e. the device may comprise one or more cryogenic jets. The cooler can provide a cold temperature of at least -100 °C. The thermal modulator 1 comprises a heater that is a resistive heater comprising heating coils 105 in thermal contact with the trapping column 5. The resistive heater can attain a hot temperature of at least 200 °C. While the heater is illustrated with six heating coils, it be appreciated that the device may contain a larger or smaller number of heating coils.

[0064] The thermal modulator may also comprise a chamber that at least substantially encloses the heater and/or cooler. The chamber may be insulated. While the illustrated device has heating coils, resistive heating may be applied in any other manner that would be known to the person skilled in the art. For example, the trapping column or the housing of the trapping column may comprise a conductive material or a conductive coating and the resistive heater may comprise applying a current to the conductive material or conductive coating.

[0065] Figure 5 illustrates a device comprising a thermal modulator 1 and a trapping column 5. The thermal modulator 1 comprises a chamber configured to substantially enclose a bath liquid 120. The chamber may be insulated. The bath liquid is in thermal communication with the trapping column 5. The chamber comprises an inlet 125 and an outlet 130 for the bath liquid 120. The inlet comprises a valve 135 that switches the thermal modulator between a hot bath liquid and a cold bath liquid. The hot and cold bath liquids may be readily selected, based on the known melting and boiling points of the bath liquids, which may represent individual liquid compounds, solutions, or mixtures of liquids. Suitable hot bath liquids include oil bath oils such as paraffin, glycerol, silicon oil or cottonseed oil. Suitable cold bath liquids include water, glycol based water mixtures (e.g. 60:40 ethylene glycol:water, freezing point -52.8ºC; boiling point 111ºC) or silicone based

fluids (polydimethylsiloxane, freezing point approximately -50ºC, boiling point approximately 120ºC). The hot bath liquid may be provided from a reservoir of hot liquid and the cold bath liquid may be provided from a reservoir of cold bath liquid.

EXAMPLES

Example 1

Experimental

a. Materials, Analytes, Solvents and Monolithic Column

[0066] Ethylene dimethacrylate (EDMA, 98%), Butyl methacrylate (BuMA, 99%), 3-(trimethoxysilyl)propyl methacrylate (γ-MPS, 98%), 2,2'-azobisisobutyronitrile (AIBN, 98%), 1-decanol (99%), toluene (99.9%), aluminium oxide were obtained from Sigma Aldrich (Zwijndrecht, The Netherlands). Methanol (MeOH), acetonitrile (ACN), acetone p-Xylene, and tetrahydrofuran (THF, > 99.8%) were purchased from Biosolve (Valkenswaard, The Netherlands). Thiourea (> 99%) was purchased from B.D.H. Chemicals (Poole, England). Sodium hydroxide (NaOH) was purchased from Merck (Darmstadt, Germany). Hydrochloric acid (HCl) (37%) was obtained from Acros (Geel, Belgium). Milli-Q water (18.2 MΩcm) was produced by a Sartorius Arium 611UV Ultrapure Water System (Goettingen, Germany). Fused silica capillary tubing was purchased from Polymicro Technologies (Phoenix, AZ, USA) (200 µm i.d., 360 µm o. d. for the monolithic column and 50 µm i.d., 360 µm o.d. for the connections). Capillary tubing was connected using IDEX Health & Science P-772 Micro tight unions. A 15cm BUMA-EDMA monolithic column was made using incomplete polymerization. This monolith is known to be a thermally stable monolith. The protocol used for preparation of the monolithic column is provided in T. B. Tennikova, F. Svec & B. G. Belenkii, *Journal of Liquid Chromatography*, 1990, 13 (1), pp 63-70 and also in Q. Ching Wang , Frantisek Svec , Jean M. J. Frechet, *Analytical Chemistry*, 1993, 65 (17), pp 2243–2248. An analyte mixture was prepared to contain final concentrations of 0.6mg/ml thiourea, 12.1mg/ml toluene, 10.5mg/ml p-xylene, and 26.3mg/ml benzophenone in 99:01 ACN:water. The analyte mixture was shaken vigorously prior to use.

b. Instrumentation

[0067] The instrumental set-up used is illustrated in schematic form in figure 2. Loading of the trapping column 5 was performed with an Agilent Technologies 1100 Series pump at 10 ul/min, equipped with a G1379A degasser. A Shimadzu CBM-20A controller recorded the UV trace from a Linear UVIS200 detector. The first valve 10 was a VICI EHMA electronically actuated valve while the second valve 11 was a Cheminert C2-2000D air actuated valve. 20 nl of sample was injected using a Cheminert 4-0344-.02 manual

injector (the injection valve 12). Back flushing of the column was done using a Shimadzu LC-10ADvp pump (pump P_2) at 10ul/min. The trapping column 5 was heated using a Waters Column heater module and cooled with a make- shift ice bath.

c. Experimental procedure

[0068] Three experimental procedures were followed to test the thermal effects on retention, the possibility of solvent switching and the focusing capability of the setup.

[0069] (i) To investigate the thermal effects on retention, the first valve 10 and the second valve 11 were each kept in position A (indicated by the dark lines) for the duration of the run. The trapping column 5 was placed in the column heater at 150 °C or in an ice bath for cooling at 0 °C. Pump P_1 was configured to pump 25% ACN in water at 10 ul/min.

[0070] (ii) To investigate solvent switching, the mobile phase of pump P_1 was spiked to contain 0.033 mg/ml Thiourea. At t=0 min the column was kept at 150 °C and the analytes were injected. At t=0.33min, the first valve 10 was switched to position B (indicated by the faded lines) and the column was inserted into an ice bath. At t=10.5 min, the second valve 11 was switched to position B to elute in a back-flush manner. The same experimental procedure was followed to explore focusing in experimental procedure (iii), except that in (iii) no additives were added to the mobile phase of pump P_1 and pump P_2 pumped a mobile phase (40% ACN in water) that had a higher elution strength than the mobile phase of P_1.

Results and Discussion

a. Thermal effects on retention

[0071] The results from experimental procedure (i) are illustrated in figure 6, where an LC trace obtained where the monolithic column was heated up to 150^0 C was compared to an LC trace obtained when the experiment was repeated at 0^0 C. At 150^0 C the retained analytes coelute at about 2 min with retention factors of ~1 (figure 6, trace 200) while at 0 °C they are well separated with retention factors of 11 for Toluene, 21 for p-Xylene and 29 for Benzophenone (figure 6, trace 201).

b. Solvent switching

[0072] Solvent switching of analytes in analytical chemistry is an integral part of many analysis methods. In 2D-LC modulation, solvent switching typically aims to transfer analytes from the eluent of the first dimension into a more compatible injection solvent for the second dimension. In order to achieve solvent switching, the differences in retention due to temperature are exploited in the present method. The trapping column is loaded and eluted in a Hot-in-Cold-out mechanism. In order to investigate the solvent switching

capabilities of the presented setup, a tracer was added to mobile phase of pump P_1. This was done to ensure that the solvent boundary between the eluents of pump P_1 and pump P_2 would be visible on the trace obtained with a UV detector. The transfer of analytes between the mobile phases would then be apparent, even though the eluents had the same organic solvent content (25% ACN in water). This introduction of tracer in the P_1 eluent shows up as a "box" on the recorded UV-trace as can be seen in trace 212 of figure 7. When a Hot-in-Hot-out mechanism is employed all analytes co-elute within the loading solvent marked with tracer (trace 210 of figure 7). When the column is cooled after loading in a Hot-in-Cold-out manner, the analytes transcend the solvent boundary, from the loading solvent containing the tracer into the elution solvent, as is indicated by trace 211, with the peaks for the analytes toluene 215, p-xylene 216 and benzophenone 217 eluting after the tracer. Optimal transfer of analytes from the loading solvent to the elution solvent was obtained when the column was cooled for 10.3min with the ice bath. Cooling for longer did not show improvement.

c. Focusing

[0073] In order to illustrate the focusing that can be achieved by the device, pump P_2 was equipped with 40%ACN in water mobile phase. The same procedure was followed as for solvent switching. After loading the trapping column 5 was brought offline with pump P_1 by switching the first valve 10 to position B and the column was inserted into an ice bath. At t=10.5min, the second valve 11 was switched to position B eluting the trapping column 5 in a backflush manner. The comparison of elution with 25%ACN and 40%ACN can be seen in figure 8, with trace 220 indicating elution with 40%ACN and trace 221 illustrating elution with 25%ACN. The figure 8 illustrates how the elution with a strong solvent (i.e. a solvent with a high elution strength) sweeps the analytes into a sharper peak when compared with an elution solvent that has the same elution strength as the loading solvent. The level of focusing that can be achieved by this method is subject to a number of factors. Under these conditions presented here, a focusing of about 37x was achieved, based on a comparison of the intensities (heights) of the analyte peaks in figure 8.

Conclusions

[0074] This example demonstrates a novel trapping device with solvent switching capabilities and focusing potential. This device is applicable to analytes that show increased retention as temperature is decreased and it therefore may be beneficial in LC modulation more generally, e.g. in 2D-LC. The utility of the device is demonstrated with three low-molecular weight model compounds. Ideally a thermal modulator device used in LC×LC would have a faster thermal response than this system, however described hereinabove are thermal modulators that would provide a faster response time. In

addition, minor modifications to the setup tested would provide a faster response time. For example, the fused silica capillary wall of the tested trapping column acts as a thermal insulator and increases the time needed for the inner part of the column to reach the lowest possible temperature. Providing a trapping column with a wall material that has higher thermal conductivity, e.g. a metal, would decrease the time.

CLAIMS

1. A device for trapping and eluting a liquid chromatography eluate, the device comprising:

a thermal modulator having a heating mode, cooling mode and thermal maintenance mode; and

a temperature control unit operationally coupled to the thermal modulator such that the temperature control unit applies the heating mode, cooling mode and thermal maintenance mode;

wherein the thermal modulator is configured to receive a trapping column located at least substantially within the thermal modulator, the trapping column configured for connection to a liquid input and a liquid output that are not located within the thermal modulator;

wherein in the heating mode the thermal modulator is configured to increase the temperature of the trapping column when fitted by at least 25°C at a rate of at least 1°C per second;

wherein in the cooling mode the thermal modulator is configured to decrease the temperature of the trapping column when fitted by at least 25°C at a rate of at least 1°C per second; and

wherein in thermal maintenance mode the thermal modulator is configured to maintain the temperature of the trapping column when fitted.

2. The device of claim 1, wherein the thermal modulator comprises a heater that is activated during the heating mode.

3. The device of claim 2, wherein the heater comprises a resistive heater.

4. The device of any preceding claim, wherein the thermal modulator comprises a cooler that is activated during the cooling mode.

5. The device of claim 4, wherein the cooler comprises at least one cryogenic liquid jet or at least one cryogenic gas jet.

6. The device of claim 1, wherein the thermal modulator comprises a chamber configured to substantially enclose a bath liquid in thermal communication with the trapping column, such that in the heating mode a hot bath liquid is introduced into the chamber, and in cooling mode a cold bath liquid is introduced into the chamber.

7. The device of claim 6, wherein the chamber comprises an inlet and an outlet for the bath liquid, wherein the inlet comprises a valve to switch between the hot bath liquid in heating mode and the cold bath liquid in cooling mode.

8. The device of any preceding claim, wherein in the heating mode the thermal modulator is configured to increase the temperature of the trapping column when fitted by at least 50°C, optionally by at least 75°C, further optionally by at least 100°C.

9. The device of any preceding claim, wherein in the heating mode the thermal modulator is configured to increase the temperature of the trapping column when fitted at a rate of at least 1.5°C per second, optionally at a rate of at least 2°C per second, further optionally at a rate of at least 2.5°C per second, further optionally at a rate of at least 3°C per second.

10. The device of any preceding claim, wherein in the cooling mode the thermal modulator is configured to decrease the temperature of the trapping column when fitted by at least 50°C, optionally by at least 75°C, further optionally by at least 100°C.

11. The device of any preceding claim, wherein in the cooling mode the thermal modulator is configured to decrease the temperature of the trapping column when fitted at a rate of at least 1.5°C per second, optionally at a rate of at least 2°C per second, further optionally at a rate of at least 2.5°C per second, further optionally at a rate of at least 3°C per second.

12. The device of any preceding claim, wherein in the heating mode the thermal modulator is configured to increase the temperature of the trapping column when fitted to up to at least 100°C, optionally up to at least 200°C, further optionally to up to at least 300°C.

13. The device of any preceding claim, wherein in the cooling mode the thermal modulator is configured to decrease the temperature of the trapping column when fitted to below ambient temperature.

14. The device of any preceding claim, wherein in the cooling mode the thermal modulator is configured to decrease the temperature of the trapping column when fitted to not more than 0°C, optionally to not more than -20°C, further optionally to not more than -50°C.

15. The device of any preceding claim, further comprising a trapping column.

16. The device of claim 15, wherein the trapping column is a capillary LC column.

17. A two-dimensional liquid chromatography (2D-LC) system, comprising a first LC column, a second LC column and a device of any of claims 1 to 16 comprising a trapping column, wherein the trapping column is located in fluid connection between the first LC column and second LC column.

18. The 2D-LC system of claim 17, wherein the trapping column has a first end, wherein in a load mode the first end of the trapping column comprises an inlet in fluid connection with an outlet of the first LC column, and in a backflush elute mode the first end comprises an outlet in fluid connection with and inlet of the second LC column.

19. A method of on-line solvent switching for an effluent from a liquid chromatography column, the method comprising:

(a) providing a device of any of claims 1 to 16 comprising a trapping column;

(b) setting the thermal modulator to a first thermal maintenance mode, wherein the temperature of the trapping column is maintained at a hot temperature;

(c) introducing the effluent from a liquid chromatography column to the liquid input of the trapping column, wherein the effluent comprises a solvent and at least one analyte;

(d) setting the thermal modulator to the cooling mode until a cold temperature is reached and then setting the thermal modulator to a second thermal maintenance mode, wherein the temperature of the trapping column is maintained at the cold temperature;

(e) stopping introducing the effluent at about the same time as, shortly before, or shortly after setting the thermal modulator to the cooling mode; and

(f) introducing an elution solvent to the liquid input or liquid output of the trapping column, such that the at least one analyte is eluted by the elution solvent, the eluted at least one analyte and elution solvent forming a modulated effluent.

20. The method of claim 19, wherein the elution solvent has a higher elution strength than the solvent of the effluent.

21. The method of claim 19 or claim 20, wherein step (f) further comprises introducing the modulated effluent to a second liquid chromatography column, or a detector, or collecting the modulated effluent.

22. The method of any of claims 19 to 21, wherein the method further comprises:

(g) setting the thermal modulator to the heating mode until the hot temperature is reached, and then repeating steps (b) – (f).

23. The method of any of claims 19 to 22, wherein the method is a back flush method and the elution solvent is introduced into the liquid output of the trapping column.

24. The method of any of claims 19 to 23, wherein the hot temperature is at least 25°C higher than the cold temperature, optionally at least 50°C higher than the cold temperature, further optionally at least 100°C higher than the cold temperature, still further optionally at least 200°C higher than the cold temperature.

25. The method of any of claims 19 to 24, wherein in the cooling mode the thermal modulator decreases the temperature of the trapping column at a rate of at least 1.5°C per second, optionally at a rate of at least 2°C per second, further optionally at a rate of at least 2.5°C per second, further optionally at a rate of at least 3°C per second.

26. The method of any of claims 19 to 25, wherein in the heating mode the thermal modulator increases the temperature of the trapping column at a rate of at least 1.5°C per second, optionally at a rate of at least 2°C per second, further optionally at a rate of at least 2.5°C per second, further optionally at a rate of at least 3°C per second.

27. The method of any of claims 19 to 26, wherein the hot temperature is at least 100°C, optionally at least 200°C, further optionally at least 300°C.

28. The method of any of claims 19 to 27, wherein the cold temperature is not more than 0°C, optionally to not more than -20°C, further optionally not more than -50°C, still further optionally not more than -100°C.

29. Use of the device of any of claims 1 to 16 as a modulator between the first and second liquid chromatography dimensions in a two-dimensional liquid chromatography (2D-LC) system.

30. A device for trapping and eluting a liquid chromatography eluate substantially as hereinbefore described with reference to, or as illustrated by, the accompanying drawings.

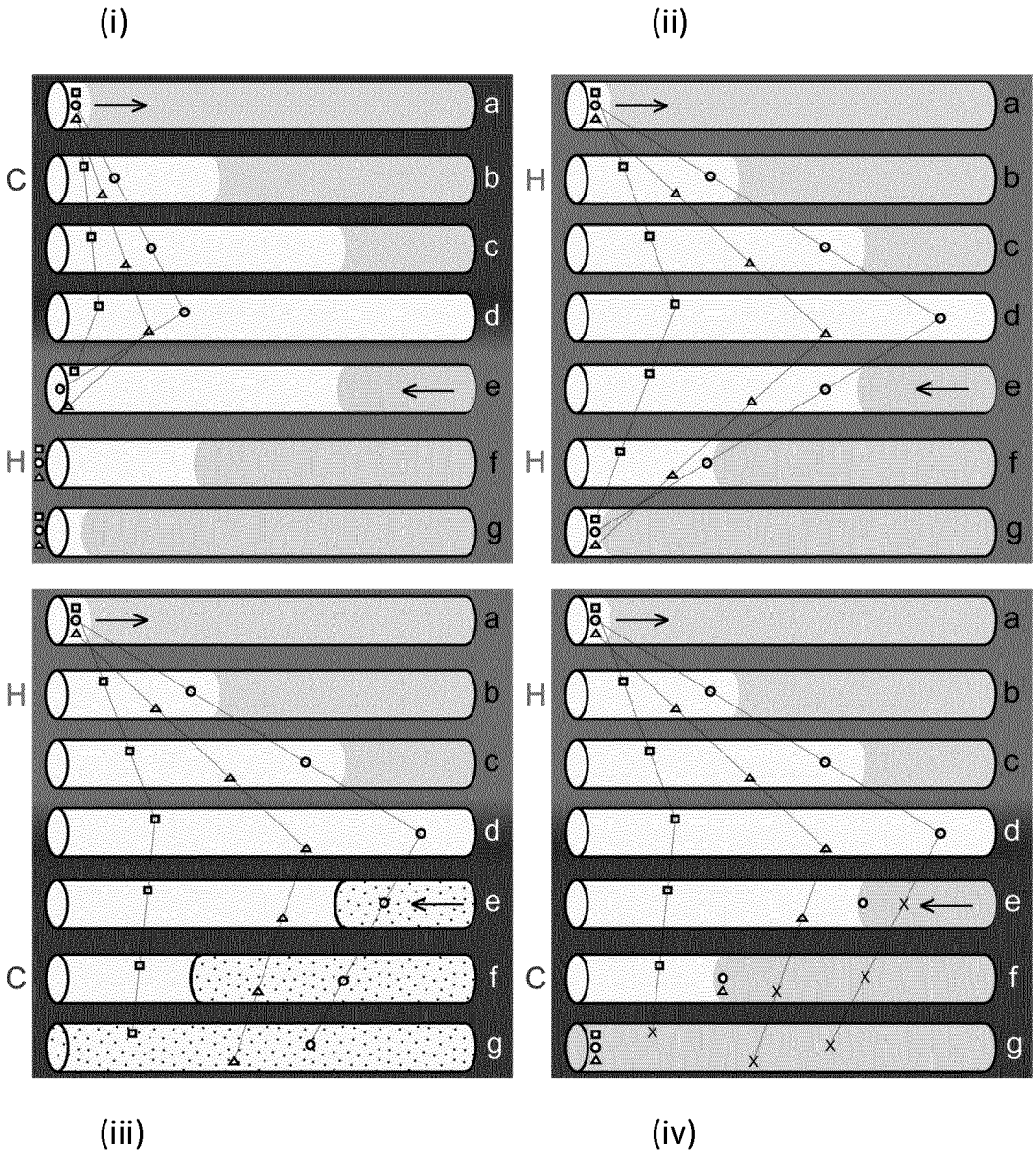


Fig. 1

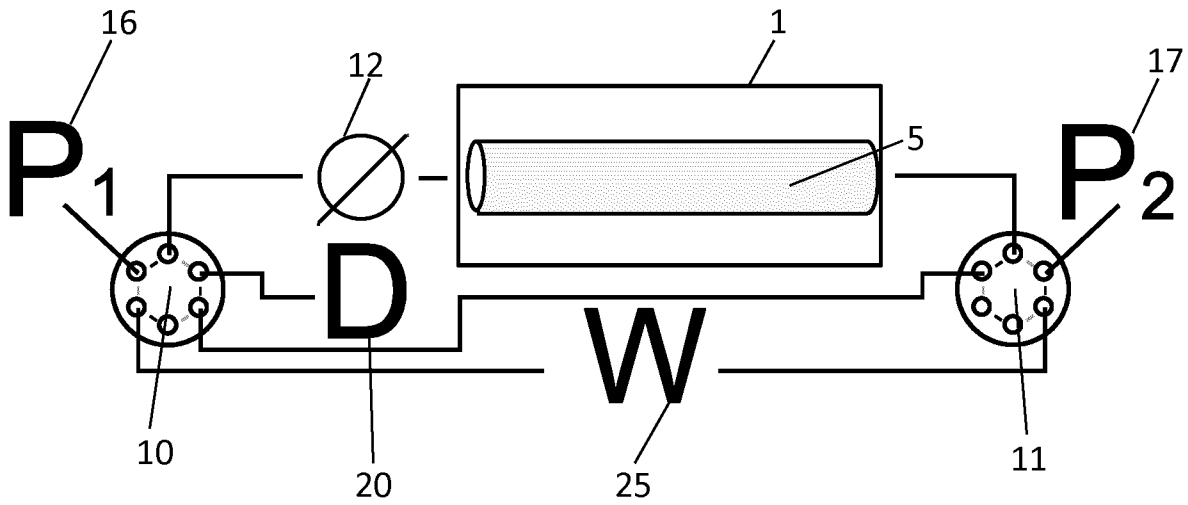


Fig. 2

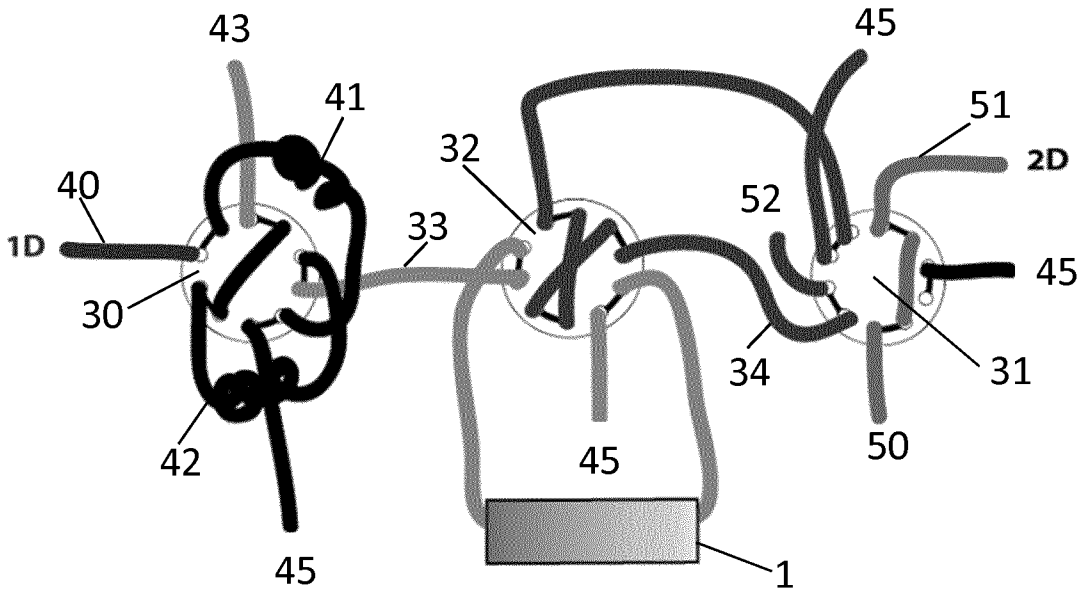

43
41
40
1D
30
42
45
32
33
45
45
51
2D
52
45
34
31
50
1

Fig. 3(a)

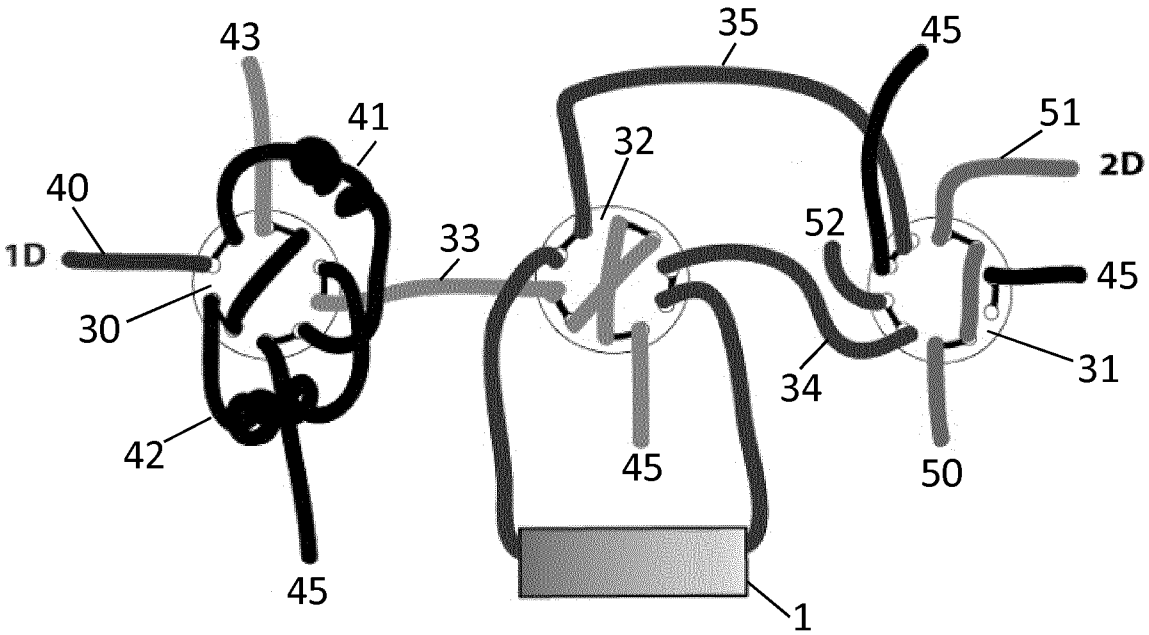

43
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1D
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45
51
2D
52
45
34
31
50
1

Fig. 3(b)

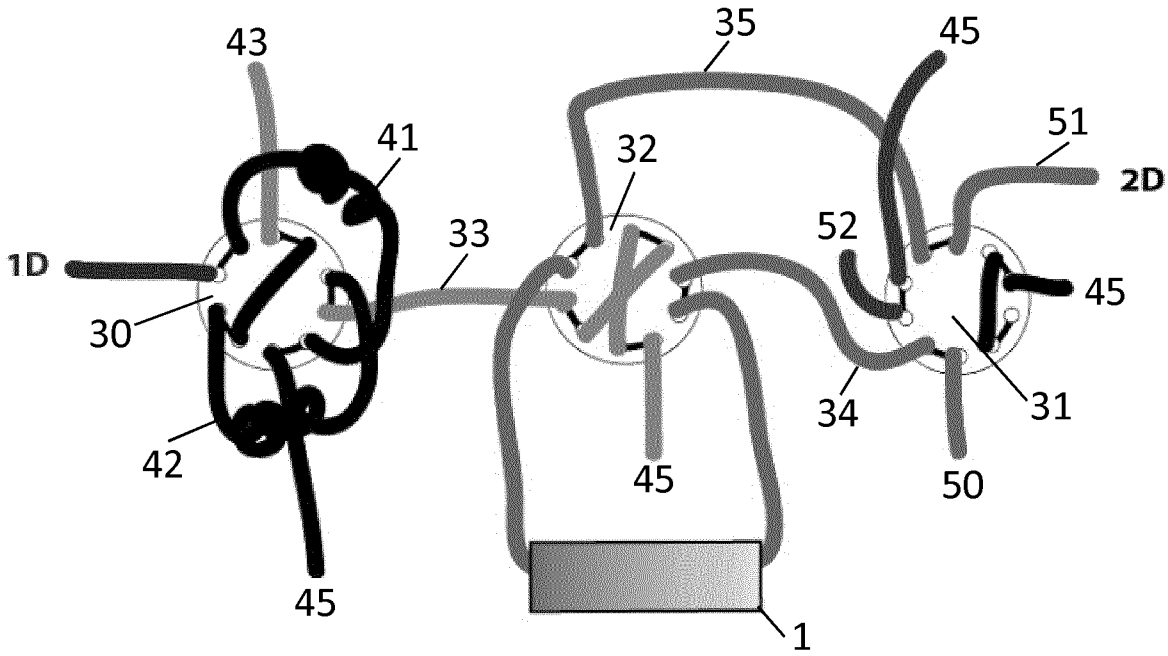


Fig. 3(c)

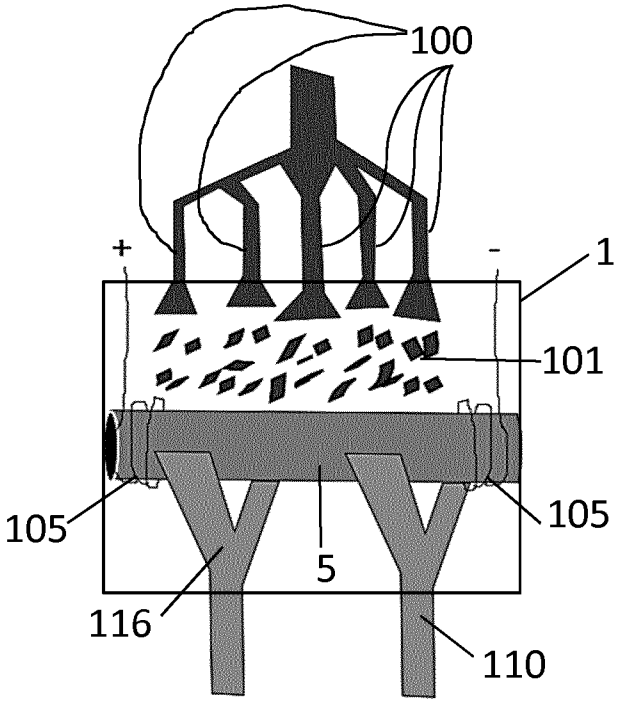


Fig. 4

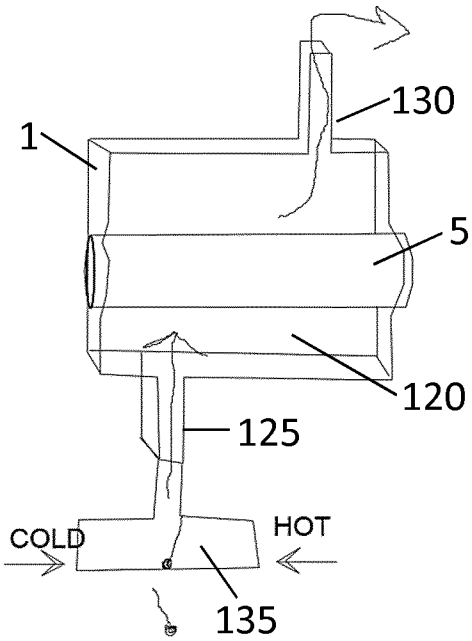


Fig. 5

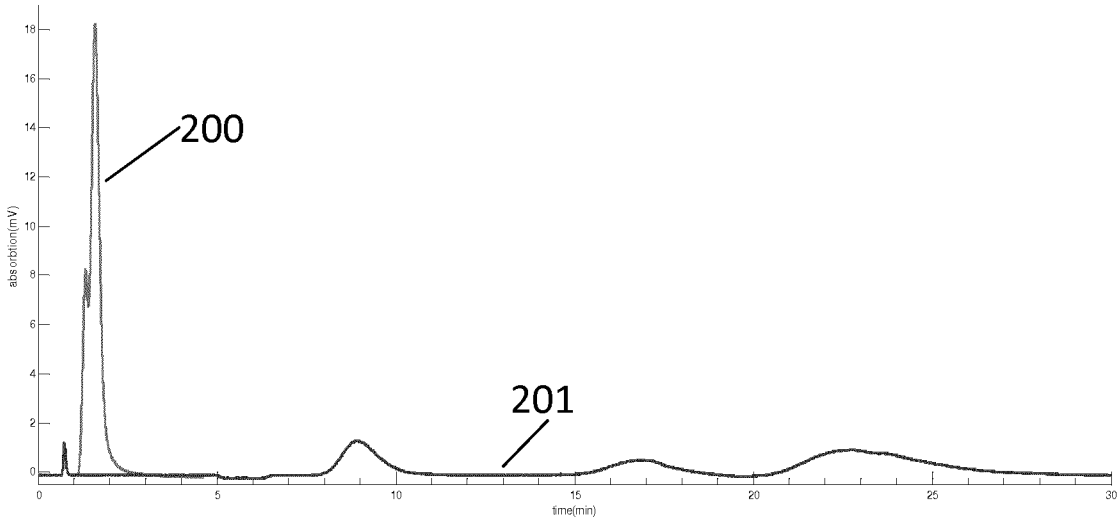

200
201
absorbtion(mV)
time(min)

Fig. 6

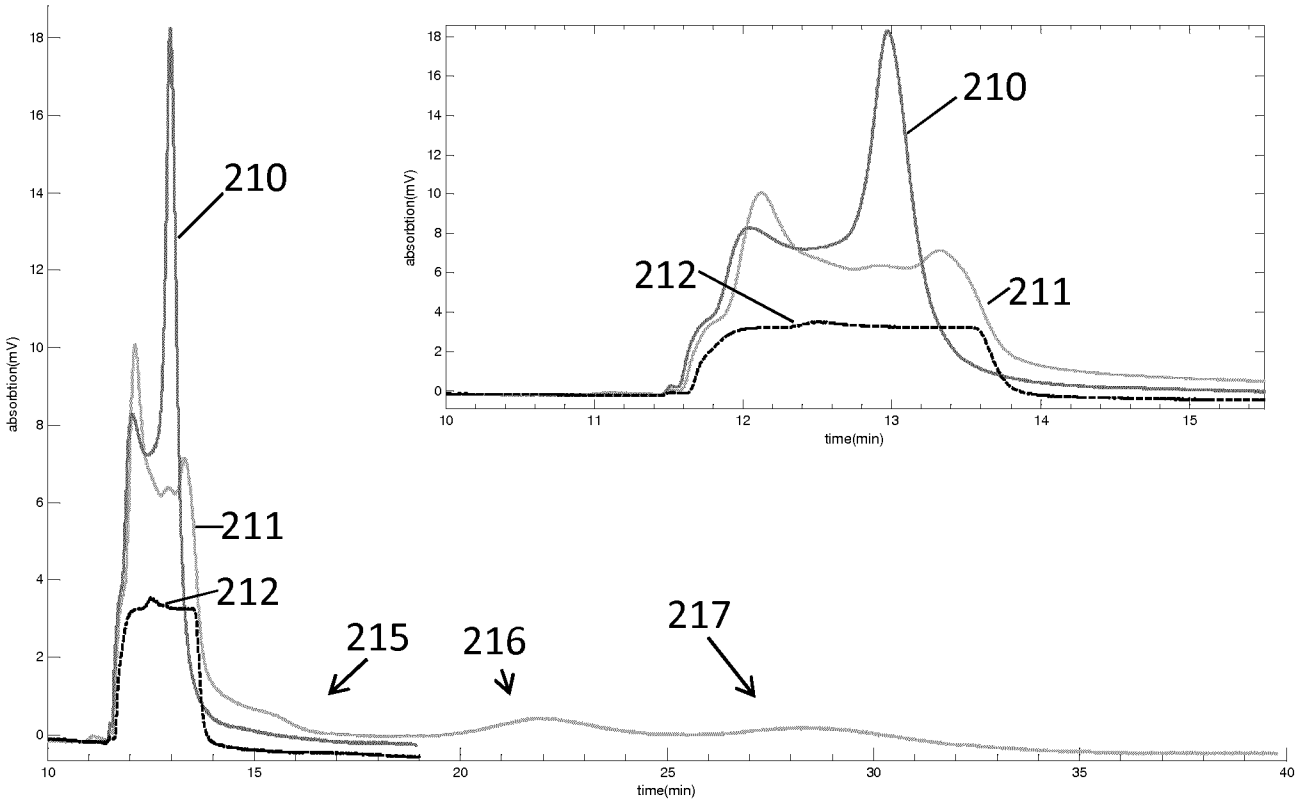

210
211
212
215
216
217
absorbtion(mV)
time(min)

Fig. 7

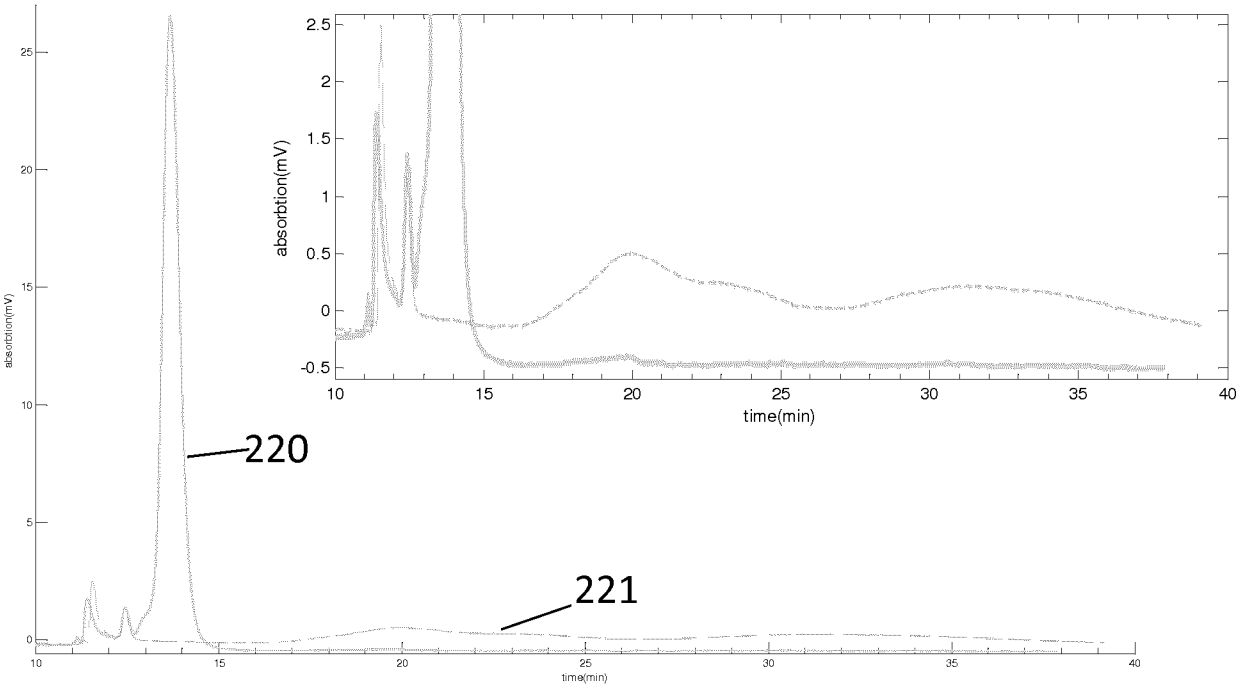


Fig. 8