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REVIEW

ROBOTICS IN BIOMEDICAL CHROMATOGRAPHY AND ELECTROPHORESIS

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CONTENTS

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LIST OF ABBREVIATIONS

AASP	Advanced automated sample preparation
CPU	Central processing unit
GC	Gas chromatography
GC–MS	Gas chromatography–mass spectrometry
HPLC	High-performance liquid chromatography
I/O	Input/output
LIMS	Laboratory information management system
MLS	Zymate master laboratory station
PC	Personal computer
PERL	Perkin Elmer robot language
RL	Rate limiting step
SPE	Solid-phase extraction
TLC	Thin-layer chromatography
TT	Total run time for each sample

1 INTRODUCTION

Recent technological innovations have profoundly influenced the practice of chromatography and electrophoresis Microprocessor-controlled instruments are now the norm Most modern chromatographs are equipped with automatic samplers for unattended analysis and with integrators and data systems for automatic data capture, integration and report generation Several devices have recently been introduced to automate sample preparation, the most laborious and error prone part of biomedical chromatographic methods One such device, the robot, promises to close the gap and to finally permit the full automation of the laboratory

In just seven years since the introduction of the first laboratory robot [1], more than 1500 robotic systems have been placed in customer's laboratories In most instances, robotic utilization appears to be carefully planned and rationally justified In a few cases [2], it appears that investment in robotics was also motivated by the fascination with robotic attributes or by the desire to render a state-of-the-art appearance to the laboratory

Gambino [3] was first in 1971 to propose utilizing the robot to automate laboratory operations. Zymate, a robot designed specifically for the laboratory was introduced in 1982 [1] Excellent tutorials, published in 1983 [4,5], introduced laboratory robotics in detail to the analytical community Since then, a significant portion of laboratory robotic reports appeared in non-refereed and, for the most part, non-scrutinized publications Most [6–10] of the latter are not even indexed for retrieval by computer searching techniques Thus far, only one book [11] on laboratory robotics has been published and was viewed as a disappointment [12] A new journal called 'Laboratory Robotics and Automation' made its debut in 1989 and it appears to accept papers that have previously been published [13,14]

This paper focuses on the robots as a useful tool for the automation of biomedical chromatography and electrophoresis methods It critically reviews the progress that has been made and speculates on future developments.

2 HARDWARE AND SOFTWARE OF ROBOTIC SYSTEMS

2 1 Attributes of commercial robots

For the purpose of this review, the robot is defined as 'a reprogrammable, multifunctional manipulator capable of moving a variety of tools and parts through a series of variable preprogrammed tasks'. This definition, which is accepted by the society of manufacturing engineers [15] and by the Robot Institute of America [16], excludes several dedicated automation devices and smart autosamplers [17–19], some of which have been referred to as robots [20].

The core of the robotic system is the arm which can move in space within a fixed working envelope to perform repetitive manual operations such as pipeting, weighing, centrifugation or chromatographic injection The arm motions are programmed by the controller, or the central processing unit, CPU, where programs are created and executed The robotic system also encompasses several specialized modules and laboratory instruments such as tube racks, centrifuges and evaporators Many of these modules need to be reached by the arm and have to be secured in a fixed location within the arm's working envelope The controller also has to be in communication with various modules, allowing the operator and the program to invoke commands (i.e., turn off and on) and collect status information (i e , centrifuge rotor position)

The attributes of several common commercial robots are contrasted in Table 1 The Zymate robotic system continues to be the most popular With its large market share, it has the largest user base and is backed by a large service and support organization and perhaps the most extensive repertoire of specialized modules and peripherals Other laboratory robots user's base is much smaller but their list of laboratory modules is increasing The movement of the Mit-

TABLE 1

ATTRIBUTES OF SOME COMMON LABORATORY ROBOTS

	Zymate II	Masterlab	Smart-arm	AlphaII+
Robot	Zymark Z100	Mitsubishi RM-501[a]	Mitsubishi RM-501[a]	Microbot Alpha[b]
Manufacturer/distributor	Zymark Corp (Hopkinton, MA, U S A)	Perkin-Elmer (Norwalk, CT, U S A)	Josco Systems (Caldwell, NJ, U S A)	Microbot (Sunnyvale, CA, U S A)
Geometry	Cylindrical	Revolute	Revolute	Revolute
Work envelope (cm)	61×56	66 reach	66 reach	46 hemispherical
Position repeatability (mm)	1 27	0 51	0 51	0 38
Motors	DC Servo	DC Servo	DC Servo	Stepper
Axis of motion	4	5	5	5
Maximum speed (cm/s)	32	40	40	129 5
Controller	Specialized, single-disk drive	IBM PC	Dedicated processor and a PC	Dedicated 6502 processor and a PC
Position teaching	Pendent/keyboard	Pendent/keyboard	pendent	Pendent/joystick
Programming language	Easylab	PERL	BASIC, Assembly	BASIC
Collision/tactile sense	Sense forces applied to motion axis	No	No	No
Grip position sense	No	Yes	Yes	Yes
Function change	Interchangeable hands	Interchangeable fingers	Custom grippers	Custom grippers
Weight (kg)	17 2	27	27	14
Payload (kg)	1 4	1 2	1 2	1 4
Approximate number of laboratory units	1400	Not known	15	50
Approximate base price (US$)	30 000	40 000	15 000	17 000
References	5,21-26	21, 22, 24-27	24, 25, 28	5, 15, 22, 24, 25, 29

[a]This basic robot, with additional modules, is also available under the name Movemaster from Seidenader Equipment (Morristown, NJ, U S A)
[b]This basic robot, with additional modules, is also available under the name MAXX5 from Fisher Scientific (Pittsburgh, PA, U S A)

subishi arm is generally faster than that of the Zymate. Its revolute geometry allows it to go over and around objects, hence reaching tight spots Furthermore, its positional accuracy (repeatability) is superior Its controller, a standard IBM personal computer (PC), permits the utilization of extensive off-the-shelf software, operating systems and instrument control and communication boards, hence facilitating the integration of the robot into the laboratory electronic environment.

2 2 Programming the robot

Upon arrival, the robot has already been preprogrammed with a small dictionary containing certain commands of motion such as arm IN or OUT, UP or DOWN, WRIST 70°, etc Starting with these initial commands, the user can instruct the robot arm to move to new coordinates This is done by the tracking pendant keys, the soft keys of the controller or the standard computer keys The coordinate positions are defined as either relative or absolute positions in space. Each new position may be given a unique name (i e , OVER·CENTRIFUGE) and all user-defined names can be stored in the reusable dictionary [24,30] The user can also create subroutines by stringing several predefined commands in any sequence The new subroutines can be given user-defined descriptive names (i.e , PICK·UP·TUBE) The subroutine names are also stored in the dictionary Likewise programs can be created (and named and stored) by linking various predefined commands and subroutines Entering any previously defined program name from the keyboard (i.e , AMINO·ACID·ANALYSIS) will cause it to be executed.

Typically, the robot arm travels along the shortest route as it moves from point A to point B All the arm joints move simultaneously. However, the speed of the movement is not necessarily the same for all the axes of motion For example, the movement of the Zymate robot is fastest along the horizontal coordinate (in/out) followed by the vertical coordinate (up/down) and is slowest for the rotary movement (clockwise/counter clockwise) This hierarchy of motion must be anticipated during the programming of various sequences of motion, lest the arm may collide with objects on its bench as it moves from one location to the next To be safe and to avoid collision, the programmer may also break a large motion into small segments, hence, forcing the arm to follow a clear path The programmer is further advised to raise and retract the arm to a safe level before any long sweeping rotation of the arm is executed [24]

Both the Zymate programming language (EASYLAB) and the Perkin-Elmer Masterlab language (PERL) are menu-driven, English-like and easy to learn. All other robots that utilize an external PC or a host computer can be programmed in any of the available high-level languages such as BASIC

An interesting programming feature of the Zymate and Masterlab robots,

the rack indexing algorithm, simplifies programming the arm positions for handling tubes in a large rack. The location of tubes in the rack need not be individually defined. The rack is defined as a matrix by driving the arm to the rack limits and by defining the number of rows and columns within the matrix. Of course, the new matrix can be named and stored in the dictionary [30]

Programming the robot is more than programming the motion of its arm to move tubes and inject samples In an integrated robotic system, all the instruments and associated laboratory devices on the robotic bench are also under the control of the robot program In the Zymate robot with its dedicated controller some specialized devices (modules) such as the centrifuge are fully integrated with the system. Control parameters and status information of such modules have already been predefined by the manufacturer and are accessible from the initial dictionary Other instruments that can be added to the system can be programmed via a Zymate module called the 'Power and Event Controller' [31]. The latter contains several isolated relay contacts for controlling devices (on/off) and several logic inputs to sense switch closures Each input or relay contact can be given a meaningful name (i e., VORTEX·MIXER·OFF) which is incorporated in the reusable dictionary In other robots that are controlled via an external PC, communication with associated modules relies on the use of standard input/output (I/O) boards for external control and status input

The Zymate dictionary of commands, subroutines and programs can be stored in the non-volatile memory of its dedicated controller and is accessible as soon as the instrument is turned on In the Masterlab and other robots utilizing an external computer with volatile memory, the reusable dictionary must be accessed from the disk drive before execution

2 3 Specialized modules

The specialized modules are the laboratory devices and equipment the robot must interact with to perform the tasks of sample preparation and analysis Ideally, the robot should utilize standard laboratory equipment, but in reality, most have to be modified for utilization by the robot For some modules, the modifications are quite simple such as wiring an additional cable to turn a pump off and on by the robot controller Other modules must be extensively modified (i e , centrifuge) or even replaced with substitutes specifically designed for robotic utilization (i e , quantitative liquid transfer) All modules requiring manipulation by the robotic arm must be secured in a fixed location on the robotic bench A brief description of some specialized modules is provided below Others will be described in the context of sample preparation and analysis steps (Sections 3 and 4)

2 3 1 Racks and dispensers

Standard tube racks may have to be replaced with racks especially fabricated for the robot Since the position of tubes is best defined in the program as a matrix, the tubes must be exactly centered and the distances between them must be exactly the same The distance between tubes must also be large enough to accommodate the robot's fingers. Because the robot's reach is limited, it was suggested that whenever possible, racks should be replaced with tube dispensers [31] Some dispensers are commercially available [32]. A simple and rugged gravity-fed dispenser which was designed to accommodate centrifuge tubes has been described [31] That dispenser can accommodate 200 tubes, yet it occupies only 240 cm^2 of the bench. By contrast, racks holding 200 tubes require ten times more of the accessible bench

2 3 2 Weighing station

Most electronic digital balances are equipped for computer control and are provided with data output boards for data transfer Hence, the robot controller can turn the balance on or off and can receive tare and weight data as required by the robotic program To be utilized by the robot, the balance pan may have to be fitted with a holder for tubes and vials Microbalances equipped with a top access door are much more accommodating of the robot's fingers The door must be motorized so it can be opened and closed under program control.

The reliability of analytical balances utilized by robots has not been good due to static effects. Placing an anti-static device containing α-particle-emitting Polonium 210 inside the balance drastically improved the reliability [33] The α-particles ionize the air inside the balance, providing a path for static charge dissipation.

2 3 3 Volumetric liquid transfer

This is one of the most frequent operations during sample preparation and chromatographic analysis Most of the manual devices for liquid transfer such as pipets, syringes and liquid dispensers, are not easily adapted for use by the robot. Further, liquid transfer by the robotic arm would be slow, significantly increasing the total analysis time An effective approach is to delegate most liquid transfer operations to a specialized module under the control of the robot controller The basic unit of the module is a programmable volumetric syringe attached to an electrically or pneumatically activated valve. In the Zymate system, the specialized module for liquid transfer is called the master laboratory station, MLS The MLS is composed of three different syringes The three-syringe plungers are controlled by three separate programmable stepping motors Each syringe is connected to a primary two-way valve One side of the valve could be a solvent reservoir and the other could be a liquid destination or another two-way auxiliary valve for branching to more than one destination [31]. The valves and the plunger motors are under program control The dis-

tance traveled by the plunger and the speed and the direction of the plunger movement are all programmable parameters There are two types of liquid dispensers The fixed dispenser is stationary To receive liquid, the robot arm brings a receiving vial to it The remote dispenser is attached to the robot hand via a long tube and is used to transfer liquid aliquots from a reservoir to various points on the bench or to aspirate liquid aliquots from any vial on the bench for transfer to another vial Lines from the MLS valves have been connected to the high-performance liquid chromatography (HPLC) injection valve to aspirate samples into the injection loop and to wash the loop afterward with solvent from an MLS reservoir [31]

3 AUTOMATION OF SAMPLE PREPARATION STEPS

3 1 Handling of liquid and solid samples

Reliable quantitative measurement is often the first and most critical sample preparation step during the analysis of liquid samples such as biological fluids The approach recommended by this author is to use the remote dispenser as a single pipet and to program the robot to wash the inside and outside of this pipet after every measurement The pipet in this case is a small piece of stainless-steel tubing attached to the robot hand and connected via a long PTFE tube to one of the syringes of the MLS During the analysis of biological fluid samples, washing the tube inside and outside with a methanol–water mixture was found to be quite effective [31]. The robot was also programmed to aspirate an aliquot of normal saline before aspirating serum or plasma samples After the sample is transferred, the saline is discarded as a partial wash to prevent sample residues from sticking to the tube and to eliminate the formation of clots

Another widely utilized approach for the quantitative transfer of liquid samples involves the placement of a volumetric syringe with a programmable plunger motor and disposable tips in the robot hand [30,34–41] The two approaches for volumetric liquid measurement have been compared [31] The disposable pipet tip approach is costly in terms of purchase price and bench space requirement for pipet tip racks and additional hands. It entails additional manual work for the purchase and the placement and disposal of the tips Tip dislodging can be stressful to the robot hand, causing deterioration of the positional accuracy of the robot [34] More seriously, the tip may attach improperly or not at all [30,40] This has required the addition of verification hardware and software [30,40] Having encountered variability during liquid transfer, many investigators are now adding a weighing module and are including a weighing step to correct for improper volumetric liquid measurement [30,39]

Solid biological samples such as animal and plant tissue represent a special

challenge These must be cut to the proper sample size and manually placed in preweighed vials and may even have to be homogenized before robotic manipulation [42,43] A domestic food blender has been modified for robotic utilization [44] and may be applicable to tissue samples.

Other solid samples such as tablets, powders and medicated animal feed have either been manually transferred to robotic vials [22,38] or poured by the robot which can automatically weigh a subsample to start the analysis [34,35,45]. The addition of a vibrating motor to the pouring hand was reported to facilitate that process

The stability of samples placed on the robotic bench for unattended analysis must be assured To minimize degradation, robotic racks containing biological fluid samples were placed in coolers containing ice [31] In another robotic installation, samples were stored in a freezer with an automatic computer-controlled door [46] The robot can open the door, remove a tube containing a frozen sample and place the tube in a heating module for thawing before aspirating a sample aliquot for analysis Labile vitamins have been stabilized during unattended analysis by the addition of antioxidants to food samples [47].

3 2 Solvent extraction, mixing and centrifugation

The use of immiscible solvents to isolate an analyte from the sample matrix is a common sample preparation step in many analytical methods for biological fluid [31] and feed samples [45] Strong mixing after solvent addition was shown to be sufficient to achieve efficient extraction Plant leaves were mixed with solvent to strip their surfaces from pesticide residues [48] Other tissue samples may require homogenization Tablets were left in solvent for a while to soften [38] and were then allowed to disintegrate by sonication before a vigorous mixing step [38,49] Vigorous mixing can best be achieved in long unstoppered tubes by vortexing [31]. Very strong vortexing action was found to be a prerequisite for reliable results [30,41,50,51] To achieve strong vortexing, programmable, pneumatically controlled holding devices [31,51] were designed to secure the tube firmly during vortexing

Many investigators resorted to the use of a capping or stoppering station before mixing by shakers [48], by vortexers [41,47] or even by holding a tube in the robot hand and moving it around [37] This approach suffers from numerous disadvantages, the least of which is the cost of complex capping modules and the utilization of valuable bench space for modules and cap racks or dispensers Because the robot lacks sophisticated tactile, force and vision sensors, reliable capping and uncapping is not possible Even infrequent leakage which is reported to occur from poorly stoppered tubes [41] can lead to disastrous results The failure to cap or uncap has been documented [41] and has necessitated the addition of other verification and control modules [41,51]

Following strong mixing with the extracting solvent, centrifugation is the recommended approach to achieve the separation of immiscible phases or to remove particulate materials from the extraction solvent Some workers have relied on filtration to remove insoluble solid components [35,38,39,47] It appears, however, that relying on disposable filters entails many of the same reliability limitations associated with tube capping and pipet tip utilization Reliable centrifugation stations are presently available for the Masterlab [52] and the Zymate [31,37,46] robots The Zymate centrifuge (Zymark Corp, Hopkinton, MA, U S A.) is particularly well designed The communication with its controller is bi-directional Centrifugation time, speed, the rotor position and a top access door are all under program control The status of any parameter can be checked at any time. The rotor is fitted with a homing motor After centrifugation, the motor directs the rotor to a predetermined position, hence, placing the desired centrifuge tube directly beneath the access door [31]

3 3 Solid-phase extraction

Solid-phase extraction (SPE) has been used over the years to isolate the analyte of interest from sample matrix components The popularity of this sample preparation technique has increased dramatically with the commercial introduction of disposable cartridges and various bonded phases. More recently, SPE has been incorporated in various robotic installations [22,36,40,41,48,52,53]

A prototype robotic SPE module [41] is shown in Fig 1 After the disposable

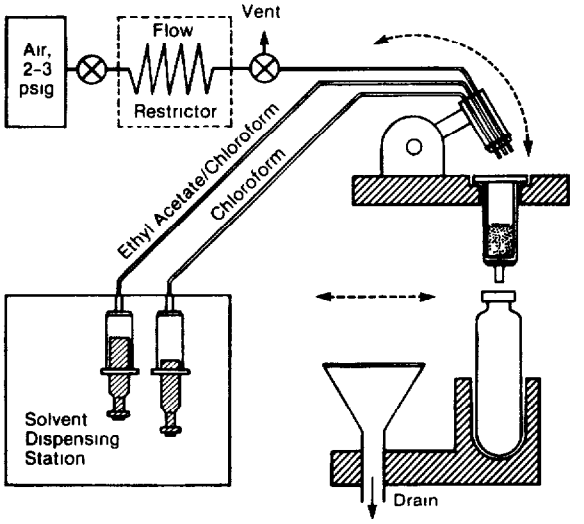


Fig 1 Schematic diagram of the solid-phase extraction station (Reprinted from ref 41 with permission)

column is placed in the module by the robotic arm, a pneumatically activated manifold nozzle is rotated to the top of the column and is sealed by a rubber O-ring Washing and eluting solvents are dispensed through the nozzle by the robot volumetric liquid transfer module To maintain an optimum solvent flow-rate through the column, positive air pressure is applied through the nozzle The SPE module also contains a pneumatically activated shuttle, placing under the column either a vial to collect the eluted sample or a funnel connected to a drainage line to discard the washing solvents [41].

Manual SPE operation is extremely rapid particularly when commercially available batch vacuum manifold devices (Analytichem International, Harbor City, CA, U S.A) are used It is unlikely that a robotic SPE system would provide higher assay throughput than that of a manual SPE method Moreover, SPE should not be the extraction method of first choice The cost of the disposable columns is prohibitive Batch-to-batch variability in commercial column performance has been documented and has led to questioning the reliability and portability of routine SPE methods [54]. Additionally, disposable cartridges have been shown to contribute additional background interferences [55]

3 4 Solvent evaporation

Several robotic modules are available to permit evaporating the extracting solvent and the concentration of the analyte All modules are standard laboratory units that have been modified to be used with the robot Reactivap nitrogen evaporator (Pierce, Rockford, IL, U S A.) has been utilized in many robotic systems [41,46]. Except for the need to incorporate a pneumatic [48] or an electrically activated nitrogen valve, no substantial modification is required The vortex evaporator (Haake-Buchler, Saddle Brook, NJ, U S A) has also been used [31,37] and is strongly recommended because it can perform several functions separately or simultaneously The simultaneous mixing and heating make the vortex evaporator an ideal module for pre-column derivatization reactions. The vortex evaporator, however, requires substantial modification [31] A vacuum pump preceded by a cold finger trap serves as the vacuum source The vacuum, heat and vortex function are under program control The vortex evaporator cover was fitted with additional weight to maintain a vacuum seal and with a special handle to permit its removal and placement by the robot hand One additional modification was needed to stop the heating block in the same location after vortexing A timer chip allows the motor to run until a flag breaks an IR beam of a photodetector Once the IR beam is broken, the timer ceases to send the drive signal and the heating block stops in the same location That mechanism was rendered extremely reliable by changing the motor and the timer every six months of operation and by tightening the motor belt once a week [50]

3 5 Prechromatographic derivatization

Chromatographic analysis is sometimes preceded by a derivatization step to permit or enhance the detection of the analyte, to improve its stability or chromatographic resolution or to render the analysis more specific. Most derivatization reactions involve the addition of liquid reagents followed by mixing, heating and/or incubation. The evaporation station, particularly the vortex evaporator, appears ideal for performing derivatization reactions by the robot. Robotic automation of derivatization reactions and subsequent gas chromatographic (GC) [56–59] and HPLC analysis [60,61] has been described. The utility of the robot in optimizing derivatization reactions is obvious. Simplex optimization techniques have been applied to maximize the yields of reactions performed by the robot [62]

4 AUTOMATION OF THE ANALYTICAL STEP

4 1 High-performance liquid chromatography

Off-line HPLC analysis is now the exception rather than the rule in analytical robotic assays In this case [30,32,36,37,44], the robot prepares the samples for analysis and places them in vials in a rack for later manual transfer to an HPLC autoinjector Some possible advantages for off-line HPLC were discussed [44] Most current robotic systems perform HPLC analysis on-line [42,45,47,61,63,64] There are basically three approaches for HPLC injection of samples by the robot The first two approaches utilize an injection valve with two positions, load and inject The injection valve positions are controlled by the robot program. In the first approach [34,41,49,60,65], the robot uses a hand containing a syringe with a motor-driven plunger (Fig. 2). The robot attaches a disposable pipet tip, withdraws the sample in the syringe and then presses the pipet tip against a conical adapter while dispensing the sample to load the HPLC injection valve. This injection approach, although reportedly yields reliable injections [65], appears to have the potential for leakage and air bubble introduction as well as memory from previous injections. Further, this approach requires a large sample volume. In one instance [60], 600 μl of sample were injected to fill a 10-μl fixed injection loop

The second and most recommended approach for on-line HPLC injection by the robot utilizes a fixed-loop injector connected to a sipper tube [31,60]. In this approach, shown in Fig 3, the robot places a vial containing the sample under the sipper tube. Suction from the liquid transfer syringe module, via long PTFE tubing, causes the sample to be aspirated into the fixed-volume injection loop After the valve is flipped to the inject position, the interiors of the sipper tube and the valve as well as the sipper tube exteriors are washed by a solvent from the liquid transfer module.

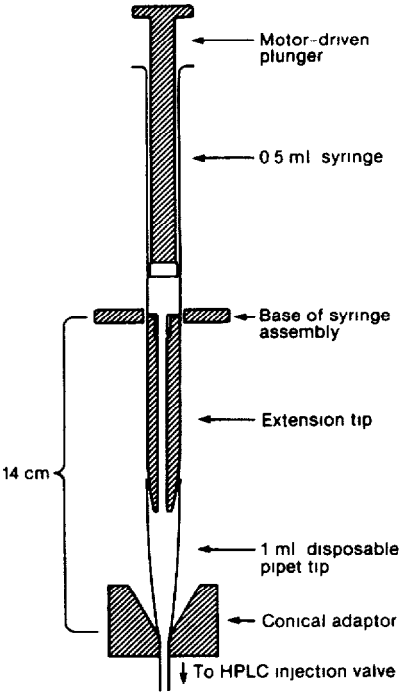


Fig 2 Schematic diagram of the syringe assembly used to load the HPLC injection valve (Reprinted from ref 41 with permission)

The third reported on-line HPLC approach combines the sample preparation and the analytical steps [40] by using a device called advanced automated sample preparation, AASP (Varian, Walnut Creek, CA, U S.A.) The device automates the HPLC injection of samples retained on solid sorbents [17] The robot conditions the AASP cartridges with solvents, transfers each sample to a cartridge, applies compressed air to drive the sample through the sorbent bed, washes the sorbent with solvents and finally transfers the loaded cartridges to the AASP for on-line elution and injection.

Robotic automation of HPLC analysis involves more than automating sample injection The availability of a dedicated robotic microprocessor affords the opportunity for instrument control and for information exchange between the robot and the chromatographic system The Zymate robot, for example, utilizes a specialized module acting as an interface between the robot and the chromatograph [31,63] In addition to controlling the injection valve position, the interface could monitor the analog output of the chromatographic detector In one application [31], the interface was used to check the integrity of the unattended analysis For this purpose, it was programmed to monitor the elution of the internal standard. If the internal standard eluted at a predetermined

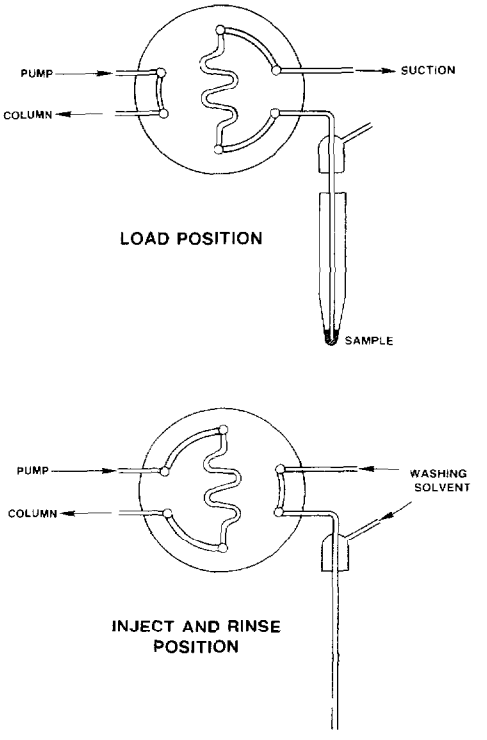


Fig 3 HPLC injection via a sipper tube and a fixed-loop injector (Modified from ref 31 with permission)

window of time, the analysis was permitted to continue If not, a problem was implied, the analysis was interrupted and corrective measures were taken

The robot controller was also interfaced to all HPLC system components to control various parameters [34,42,64]. Interfacing the HPLC pumps and valves for solvent and column selection allowed turning the pump off and on, altering the solvent composition and flow-rates, selecting the optimum column for a particular analysis, performing multi-dimensional analysis and the continuous monitoring of the column back-pressure Washing the system and automated orderly shutdown after the analysis is complete can also be performed All these parameters are under program control and the status of each parameter could provide a basis for decision-making software Likewise, interfacing the HPLC detector permitted selecting the optimum wavelength and attenuation for a given analysis and activating the detector's autozero function with each wavelength change In one application [64], a fluorescence detector was positioned downstream from a UV absorption detector, hence providing the additional flexibility of selecting one detector or the other

4.2 Gas chromatography

Sample injection into the gas chromatograph requires positional accuracy far exceeding that possible by most laboratory robots. The Zymate robot, for example, could be off by as much as 2 mm. Hence, direct syringe injection by the robot arm is not likely to be a reliable approach to automate the analytical step. Several robotic installations relied on off-line GC analysis [25,30,51]. In this case the robot is programmed to prepare the samples, place them in vials and cap the vials for later manual transfer to a GC autosampler.

To facilitate on-line direct GC injection, a cone-shaped needle guide was installed on the top of the GC injector [58]. More reliable on-line GC analysis requires a dedicated autoinjector on the robotic bench [38,66]. After sample preparation, the robot transfers the sample to an autoinjector vial, caps and places the vial in the autoinjector and finally commands the chromatographic system to proceed with the analysis [67].

4.3 Thin-layer chromatography

Thin-layer chromatography (TLC) is the simplest of all chromatographic techniques and it would appear that simple dedicated devices could be developed to automate the potentially time-consuming plate spotting. However, at least two published reports described using a robot to automate that step [5,68]. Automating the development step was also described and has required the development of a specialized robotic hand for plate transfer and a specialized TLC chamber [68].

4.4 Electrophoresis

Robotic automation of DNA electrophoresis has been described [69]. A feedback mechanism was utilized to maintain the maximum allowable voltage and to keep the temperature constant. An adjustable end point detector was utilized to conclude the electrophoresis. The robot arm was also programmed to move the gel to various stations for additional experiments.

5 IMPLEMENTING A ROBOTIC SYSTEM

5.1 Design considerations

A well designed robotic system is one that matches the capabilities and limitations of the robot to the functions to be performed, as well as to the expected sample load. In a quality control laboratory environment, the sample load may justify designing a dedicated robotic system to perform the same analytical

method continuously In a research laboratory, a flexible system for performing numerous assays, with rapid switchability from one to another, may be desired

Contrasted against humans, the robot, through its microprocessor, can keep track of numerous timed operations simultaneously On the other hand, the robot sensory inputs are absent or rudimentary and the reach of its arm in space is quite limited All items needed for a particular assay and requiring robotic manipulation must be placed within the reach of the arm in the robot work envelope. Efficient use of the robotic bench is an obvious design goal Whenever possible, items with large space requirement (i e , tube racks) ought to be replaced with substitutes with lower space requirements (i.e , tube dispensers)

It takes time to secure a module on the robotic bench and to teach the robot its location Ideally, in flexible systems where rapid switchability from one assay to the next is desired, the robot work envelope must accommodate not only items for a given assay but also items needed for most assays [31] In an attempt to facilitate switching between assays, Zymate Corp introduced the PyTechnology system In this system, hardware associated with a given analytical operation is mounted on a wedge-shaped platform The platforms can be mixed and matched around the robot as needed for an assay Because the hardware positions have been preprogrammed, the system set up is reportedly rapid [70]

Several investigators utilized innovative approaches to expand the reach of the robotic arm The arm was placed on rails and programmed to commute to various bench destinations, and was provided with a pneumatic latching system to lock itself in place once the desired destination is reached [71] The robot was also placed on wheels and was manually moved from one bench to another to perform a different assay [2] Some modules were equipped with transport devices such as wheels and tracks These modules were placed outside the work envelope. When needed, the robot grasps a handle and pulls the desired module into reach [71]. Also, a vial transport system was utilized to move the samples from one robotic system to another to complete the analysis [72].

Another obvious design goal is to assure reliable performance during unattended operation. Although the robot can be made to emulate most manual steps, some steps (stoppering tubes, attaching disposable filters or pipet tips) require the benefit of touch, force or visual sensory information Direct transfer of such steps to robotic operations increases the potential for failure It is advisable in such cases to devise more reliable approaches to achieve the same operations [31] Nevertheless, to avoid costly and time-consuming revalidation, some regulatory methods may have to be performed without any alteration [30,38]

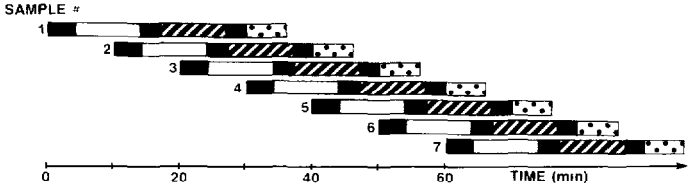
5 2 *Assay modification*

The above mentioned design considerations may dictate some alteration of the manual method to achieve reliable automation Few additional changes may be required Some assays were downscaled [35] or upscaled [31,51] to accommodate the robot fingers or the standard glassware, syringes and other equipment utilized by the robot Corrosive reagents and liquids likely to salt out or form emulsions are not recommended Obviously, during unattended analysis, flammable and explosive solvents are to be avoided Manually it may be possible during solvent partition steps to quantitatively transfer each immiscible liquid phase But, in robotic applications, depending on the tendency to form emulsions, a small fraction of each phase (0 1–0.2 ml) may have to be left behind

5 3 *Serial operation*

In the serial operation mode, one step (i e , GC analysis) is performed on one sample at a time In the batch mode, one step (i e , centrifugation) is per-

SAMPLE FLOW DURING EFFICIENT SERIAL ANALYSIS



ASSAY THROUGHPUT

	Required Time min	Waiting Time min	Total Time min
Steps requiring robotic movements (4 min for extraction steps leading to centrifugation, 3 min for liquid transfers leading to evaporation and 3 min for reconstitution and HPLC injection)	10*	—	10
Centrifugation	7	3	10
Evaporation	7	3	10
HPLC Run Time	6	4	10
Total Time for Each Sample (TT)	30		40

*Rate limiting step (RL) = 10 min
Total time for n samples = (RL X n) + (TT – RL)

Fig 4 Sample flow and assay throughput during robotic serial analysis (Modified from ref 31 with permission)

formed on several samples simultaneously In manual assays, batch mode is generally more efficient The robot, on the other hand, can be extremely efficient in the serial mode Its multi-tasking microprocessor can keep track of numerous steps running simultaneously, each on one sample During serial operation, the total analysis time for every sample from beginning to end is constant This is particularly advantageous when dealing with unstable derivatives or analytes. Another advantage is that the analysis result on the first sample is obtained early, hence providing an integrity check and an opportunity to modify the system for subsequent samples [31] Sample flow during robotic serial analysis is demonstrated in Fig 4

5 4 Integration to achieve full automation

Integrating sample preparation robots, on-line chromatographic analysis and the laboratory host computer and information management software permits the full automation of the chromatography laboratory. Layouts, electrical–mechanical connections and/or communication protocols have been described for interfacing robots with GC systems [66], HPLC systems [34,52,67,73], Apple IIC PC [74], IBM PC [75,76], VAX 8600 host computer and laboratory information management system (LIMS) [76] and Hewlett-Packard 1000 and 3357 LIMS [34,75]. Communication between the robot and the chromatography system initiates data integration and allows optimization of the chromatographic parameters Communicating sample integration results to the robot provides the opportunity for real time unattended decisions to reassay or to modify the sample preparation scheme Samples could be presented to the robot in a predetermined order and communication with the laboratory LIMS provides sample identification and assay information Otherwise, samples may be provided with bar code labels [67,75–77] and presented to the robot at random The robot, through its bar code reader, accepts the sample identification information and transmits it to the host computer to receive assay instruction Chromatographic data transfer to the laboratory computer permits performing arithmetic and statistical calculations, the automatic generation of analytical reports and the incorporation of the analytical results into the laboratory data base

6 PERFORMANCE OF ROBOTIC SYSTEMS

6 1 Quality of data

There appear to be at least two misconceptions concerning the quality of data produced by the robot The first is that the accuracy and precision of robotic assays should be better than those of manual methods [47,78] The second misconception is that robotic assays are more portable from one laboratory to another and, as long as the same robotic systems are employed, do not need to be revalidated [42,78,79] This is allegedly so because the robot "separates chemistry from laboratory techniques" [78,79] In reality, robotic

systems need to be carefully scrutinized and frequently validated Except where the analyte or its derivative is unstable, there is no theoretical basis to expect robotic sample preparation to yield higher data quality than manual methods For example, the robot's motorized syringes for quantitative liquid transfer are not always the most precise. The syringe stepping motors and valves may be affected by voltage fluctuations This necessitated the addition of a constant-voltage transformer [39] Leakage and air bubbles may go unnoticed during unattended analysis A slight drift in the robot hand position or a slight imperfection in the syringe tip or the glassware may result in failure of the syringe or pipet tip to touch the inside wall of the receiving vial This could lead to imprecise liquid delivery Many recent robotic installations incorporate a microbalance to check critical quantitative liquid transfers by weighing [30,39]. Also the same robotic module does not always perform the same in different laboratories. For example, the precision of the Zymate 1-ml syringe was reportedly better than specifications in one laboratory [40] and well outside specifications in another [80]

Many investigators report robotic data quality similar to that of manual methods [35,38,41,81] Some report improvement in accuracy and precision over the manual methods [30,40,59] In a few cases the claimed improvements were not substantiated by careful analysis of the actual results [40]

Obtaining high quality data from robotic systems requires frequent maintenance and validation [82]. We usually fortify blank samples with known concentrations and disperse them among unknown samples before analysis by the robot. These fortified samples provide a continuous check on the quality of robotic data

6 2 *Sample throughput*

Common laboratory robots are slower than humans Nevertheless, the robot is generally capable of higher sample throughput due to the ability to operate for more than one shift. Estimates of robotic assay throughput range from equivalent [46] to over five-fold [22,45] higher than that of manual methods Robotic assay throughput is dependent on the assay rate-limiting step (RL) and to a much lesser extent on the total run time per sample (TT) If RL is 10 min, one new sample can be started every 10 min during serial operation (Fig 4) The total run time for n samples equals $(RL \cdot n) + (TT - RL)$ Increasing the throughput of a robotic assay can be achieved by reducing the time for RL [31] If, for example, RL is the chromatographic run time, increasing the sample throughput can be accomplished either by faster chromatography or by the utilization of more than one chromatograph by the robot

6.3 *Biomedical applications*

Reported robotic biomedical applications include the analysis of therapeutic drugs in biological fluids by HPLC [31,36,37,40–42,46,50,81], quantitative analysis of drugs of abuse by gas chromatography–mass spectrometry (GC–MS) [77], HPLC analysis of medicated feed [22], dosage form analysis by HPLC [34,44] and by GC [38], HPLC analysis of biosynthetic insulin in fermentation broth [75], quantification of organic constituents in wastewater by GC and GC–MS [51], HPLC analysis of vitamins [47] and of carbohydrates [39] in foods, derivatization for amino acid analysis by HPLC [60,61] and by GC [57] and the purification and isolation of synthetic DNA by polyacrylamide and horizontal submarine gel electrophoresis [69,83]

7 ADVANTAGES

The utilization of robotics in the chromatography laboratory entails several advantages and opportunities, some of which are not easily quantifiable in financial terms The robot can enhance laboratory safety by working with radioactive and infectious samples and with toxic chemicals and reagents [75] Because the analysis time can be constant for every sample, some improvements in the data quality is possible when dealing with unstable analytes and derivatives

In certain applications, well designed robotic systems are very productive and can allow the laboratory to increase the sample throughput without a corresponding increase in manpower The robot can free skilled personnel from mundane and repetitive tasks and can allow their utilization in more challenging assignments [31,35,37] Some manpower savings have been reported [22] It should, however, be pointed out that the robot requires some additional manual tasks (set up, maintenance and trouble-shooting) that are not normally performed by laboratory personnel [31,59,82] Moreover, the involvement of an experienced scientist is required during the initial stage of robotic design and set up That stage could take from six months [31] to two years [59] Several organizations concluded that successful robotic implementation requires dedicated part-time or full-time specialists [30,49,70,84] The additional manpower required by robots has not always been considered in published reports highlighting the return on investment in robotics

8 FUTURE TRENDS

At least for the immediate future, and despite some expressions of concern [85], chromatographers and analytical chemists are not in danger of being replaced by robots Nor will the laboratory be completely robotized any time soon Furnishing laboratory robots with humanoid attributes, such as response

to spoken commands [86,87], has been attempted or anticipated The market will, however, favor developments that are more in tune with the realities of the laboratory A significant fraction of the tasks presently executed by robots can best be performed by simpler and more reliable dedicated instruments These instruments may not necessarily involve robotic technology and can be made easier to set up, use and program Some such dedicated instruments have just begun to be introduced [18–20] and more developments and refinements in this area are expected in the near future

More complicated applications will, of course, continue to require flexible robots and will demand enhancement in efficiency, reliability and sophistication of laboratory systems Future enhancements will involve software and hardware developments Advanced and easier to program visual, tactile and force sensory devices need to be developed and incorporated to improve reliability and flexibility of laboratory robotics The market for sophisticated flexible laboratory robots is not very large Most of the significant technological robotic advances are, therefore, more likely to be developed for purposes such as automated manufacturing Many will be borrowed and incorporated in laboratory systems

The integration of robotics, other automation tools, laboratory computers and LIMS has provided opportunities for unattended chromatography method development [25,45,88] and for the consideration of laboratory expert system development [58,70,74,86,89,90] Interest in developing robotic-based expert systems will continue

9 SUMMARY

The ideal laboratory robot can be viewed as "an indefatigable assistant capable of working continuously for 24 h a day with constant efficiency" The development of a system approaching that promise requires considerable skill and time commitment, a thorough understanding of the capabilities and limitations of the robot and its specialized modules and an intimate knowledge of the functions to be automated The robot need not emulate every manual step Effective substitutes for difficult steps must be devised The future of laboratory robots depends not only on technological advances in other fields, but also on the skill and creativity of chromatographers and other scientists

The robot has been applied to automate numerous biomedical chromatography and electrophoresis methods The quality of its data can approach, and in some cases exceed, that of manual methods Maintaining high data quality during continuous operation requires frequent maintenance and validation Well designed robotic systems can yield substantial increase in the laboratory productivity without a corresponding increase in manpower They can free skilled personnel from mundane tasks and can enhance the safety of the laboratory environment The integration of robotics, chromatography systems

and laboratory information management systems permits full automation and affords opportunities for unattended method development and for future incorporation of artificial intelligence techniques and the evolution of expert systems

Finally, humanoid attributes aside, robotic utilization in the laboratory should not be an end in itself The robot is a useful tool that should be utilized only when it is prudent and cost-effective to do so

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