ISSN : 0973-6204

PHARMBIT

INTERNATIONAL JOURNAL

Vol. XV No. 1 Jan-Jun, 2007

The Official publication of
PHARMACEUTICAL SOCIETY
DEPARTMENT OF PHARMACEUTICAL SCIENCES
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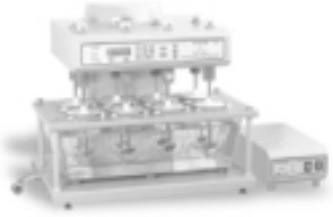

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Message

I am very glad to know that, like previous years, the **Pharmaceutical Society** of our institute has taken up the endeavour of publishing their biannual publication, **"PHARMBIT"**, for this year also I hope that the publication would be enriched by contributions both from the student community as well as by articles from the eminent personalities in the discipline of pharmacy including teachers of our Institute. The basic object of the magazine should be to make people aware of the activities of the Department of Pharmaceutical Sciences of our Institute.

I take this opportunity to thank all my students and colleagues who had taken up this commendable job of publishing this in-house magazine.

I wish the publication to become a regular annual event of the Society.

(Dr. S. K. Mukherjee)
Vice-Chancellor

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Message

Dear Dr. Gupta,

I am happy to note that you are the Editor of PHARMBIT - a Biannual publication of your Institute.

I extend my warm greetings to you and wish all the success for your efforts in bringing out a quality journal.

With regards,

Yours Sincerely

(Prof. Chandrakant Kokate)
Vice-Chancellor
&
Ex- President,
Pharmacy Council of India,
IPCA and IPA

President's Message

Very hearty greeting to all our members, teachers, scholars, student and well wishers.

We are all aware that our society is going through a massive change in its healthcare needs and provisions. We in the profession of pharmacy in India can respond to changes in the healthcare system, in part by making **Pharmaceutical Care** as mission. Pharmaceutical care is an evolutionary and revolutionary way of practicing pharmacy. It requires complete rethinking about how pharmacists in India have traditionally worked, so far. Pharmaceutical care is far more complex and offers many more challenges and opportunities. Community pharmacists should take the responsibility for preventing and solving drug-related problems and optimising drug therapy. Their responsibilities should not end by handing over drugs to patient or when the patient leaves the pharmacy.

Therefore assessing, monitoring, documenting, care and progress and follow-up care are integral parts of providing pharmaceutical care.

Good pharmacy practice (GPP) is the only answer for providing pharmaceutical care. GPP guidelines aim to set standards for practice of pharmacy as a profession in India. Standards play an important part in the measurement of quality of service. GPP guidelines have been documented with the understanding and acceptance that the conditions of pharmacy practice may vary between different areas within the country. However, it will be individual pharmacists and pharmacies who will have to decide what is the highest level of service that can be provided by them and achieving, it will be their professional decision. A certain level of commitment to change will be the essential prerequisite for adoption of these guidelines. The objective of framing and implementing the GPP guidelines for India, is that over the next few years, all Community pharmacies in India should achieve these standard of practice. These standard can be achieved through continuous education training and perseverance of the Community Pharmacists as well as the professional organizations. Time has come in India to change the product oriented approach to a patient oriented approach with pharmaceutical care as the ultimate goal of pharmacy practice.

Pharmacists can make a difference in the rising apathy towards both mental and physical well-being of the patients. We need to change perception of the role of pharmacists among other members of the health care team and raise public awareness of the added value we can offer. It is time to expand our services beyond the conventional responsibilities.

Dr. D. Sasmal
President, Pharmaceutical Society
Prof. & Head, Dept. of Pharmaceutical Science

From the Editor's Desk

It is a matter of great pleasure to bring out upgraded edition of "**PHARMBIT**"as scientific journal having **ISSN Registration**. Pharmbit is actually official publication of 'Pharmaceutical Society', which was formed 30 years ago when I have been passing out from this institute. Becoming editor and doing it's upgradation are proud work for me. From this year Pharmbit has been made biannual publication as reffered Pharmceutical journal. Accordingly new Editorial Advisory Board of Pharmbit has been constituted having eminent scientists of National & International repute. Further 'Instructions to Authors' were released while inviting articles from teachers, scientists, and writers of National & International level organisations and Institutions. It is well known fact that scienticfic journals must provide articles/ papers of high calibre,high standard,updated scientific nature, latest findings and creative research work for reference purpose as well for their readers. The scientific paper upon publication are being accepted as scientific facts. Hence after very strict and peer review of manuscripts by me and reviewers, the papers/ articles were selected for publication in this edition.Further the journal requires commited, dedicated, honest, and devoted group of personnel in the shape of editorial board members, reviewers, supporters, well wishers and editor.

As an editor of a journal, my intention is always to create readers. The professionals must involve themselves in reading the scientific journals, which will enrich them with latest trend in research and updated information related to profession. From this column I would appeal all faculty members to acquaint their students about importance of reading of journals then only overall growth of profession as well as high caliber research work would be continued and be reflected in the coming days.

As an editor of this journal, I am thankful to all authors, reviewers, Editorial Board Members, advertisers, well wishers, Head, department of Pharmaceutical Sciences, Vice -Chancellor, BIT, Mesra and Vice-Chancellor Emeritus, BIT,Mesra for their support and encouragement in bringing out this edition of PHARMBIT. Lastly I invite suggestions from readers and well wishers to improve the PHARMBIT further.

Dr R. N. Gupta

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MASS SPECTROMETRY IN PHARMACEUTICAL ANALYSIS

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Department of Pharmaceutical Analysis,
National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab

INTRODUCTION

Over the last few decades, mass spectrometry (MS) has emerged as a front-runner technique in pharmaceutical analysis, covering both qualitative and quantitative aspects. In fact, the area of use of MS is increasing at such an unprecedented rate that new applications are getting developed almost on daily basis. Coupled to it is advancement in the instrumentation, which is trying to keep pace with the ever increasing demands on sensitivity and throughput, guided at times by the increasing stringency of regulatory requirements.

Of the many analytical techniques used in drug discovery and development, MS is employed extensively for characterization and analysis of a wide range of biological and chemical entities[1]. Because of the enhanced sensitivity, selectivity, and ease of automation that is available with liquid chromatography (LC) coupled with MS (LC-MS and LC-MS/MS), a large number of absorption, distribution, metabolism and elimination (ADME) assays with high throughput have been incorporated into drug discovery programs[2]. In particular, tandem MS/MS has opened an avenue for broader and newer applications. Utilization of higher mass resolution and sensitivity in quantitative assays, a wide range of ionization methods, possibility of single ion monitoring (SRM) or multiple reaction monitoring (MRM) and availability of a variety of sample processing/clean-up methods has expanded the implications of MS even in the pharmaceutical industry. Accelerated advancements in this field are also adding worthwhile milestones in biotechnology, as well as various "-omics" (proteomics, lipidomics, metabolomics, etc)[3].

It is thus of importance for a pharmaceutical scientist to understand the variety of MS systems available in the market, including their areas of applications.

TYPES OF MASS SPECTROMETERS

Broadly, any mass spectrometer consists of three devices associated with three fundamental steps in MS analysis:

- An ion source to create gas phase ions of the analyte and its species.
- A mass analyzer which separates the mass to charge ratio (m/z) of the ionized analyte based on their mass to charge ratio (m/z).
- A detector, which measures the quantity of each mass to charge ratio (m/z).

A large variety of all the three devices are available. They are discussed in more details below.

1. IONIZATION SOURCES

Electron impact (EI)

A beam of electrons passes through a gas-phase sample and collides with neutral analyte molecules (M) to produce a positively charged ion or a fragment ion[4]. This ionization is of harsher type. Generally energy provided by electrons, almost equivalent to 70 eV, suffices to exceed the bond dissociation energy of most of the covalent bonds present in the organic molecules. The method is applicable to all volatile compounds (>103 Da) and gives reproducible mass spectra, with fragmentation to provide

structural information. It can be used in both positive as well as negative modes, depending on the chemical structure of the analyte.

Chemical impact (CI)

Ionization begins when a reagent gas (R) is ionized by electron impact and then subsequently reacts with analyte molecules (M) to produce analyte ions[5]. It is a softer technique than EI. So it provides molecular weight information, in addition to the fragmentation. Commonly used reagent gases are hydrogen, isopropane, ammonia and nitrous oxide. An important modification of CI, which is applied for the orthogonal use with HPLC, is to create ions at atmospheric pressure. The technique is referred to as atmospheric pressure chemical ionization (APCI). In this, a flow of liquid is induced to form a spray in a pneumatic (usually nitrogen powered) nebuliser. The emerging plume of liquid droplets is directed towards a corona discharge electrode that is maintained typically at 1 ± 3.5 kV[6].

Fast Atom Bombardment (FAB)

The analyte in the liquid matrix, like glycerol, is bombarded with a strong current of particles, e.g., Ar, Xe or Cs at energies of 4-10 KeV. The particles are accelerated by electric potential to yield high speed beam of ions of the analyte[7]. This is a soft ionization technique and suited for the analysis of low volatile species, typically producing large peaks for the quasi-molecular ions [(M+H)+ and (M-H)], along with structurally significant fragment ions, dimers as well as the cluster ions.

Field Ionization (FI)

Equipotent electric lines, generated by employing a high electric potential, result in crowded field around the needle tip[8]. The intensity of electric field is maxium at the point of the tip where ionization occurs. In FI, the sample is heated in the vacuum to volatilize onto an ionization surface. FI is suited for use with volatile, thermally stable compounds. Sometimes, a FI source is modified to be used in desorption mode, suited for non-volatile and/or thermally labile substances, where sample is placed directly onto the surface (dipping emitter in an analyte solution) before ionization. This is called field desorption (FD) ionization.

Electrospray Ionization (ESI)

In this technique, ionization takes place at atmospheric pressure and is considered to be soft ionization method. A solution is nebulized under normal pressure and exposed to a high electric field to create a charge on the surface of the droplet (Figure 1a). Droplet size rapidly diminishes through solvent vaporization, producing multiply charged naked ions. It is additionally applicable to thermally labile, high molecular mass substances, i.e., oligonucleotides, proteins, synthetic polymers, etc.[9] The repulsion forces between the charges increase until there is an explosion of the droplet. Multiply charged ions are obtained, depending on the chemical structure of the analyte[10]. Apart from the conventional ESI sources, modern mass spectrometers are also furnished with micro-ESI or nano-ESI ionization modes, which are more efficient in terms of their size, flow rate and ionization efficiency.

Atmospheric Pressure Photoionization (APPI)

APPI is a modified APCI source, wherein the corona electrode is replaced by a UV lamp and hence photons are used to ionize gas phase molecules[11]. In APPI, vaporized sample passes through ultra-violet light (krypton light source emitting at 10.0 eV and 10.6 eV). It has become an important ionization source because it generates ions directly from solution with relatively low background due to high

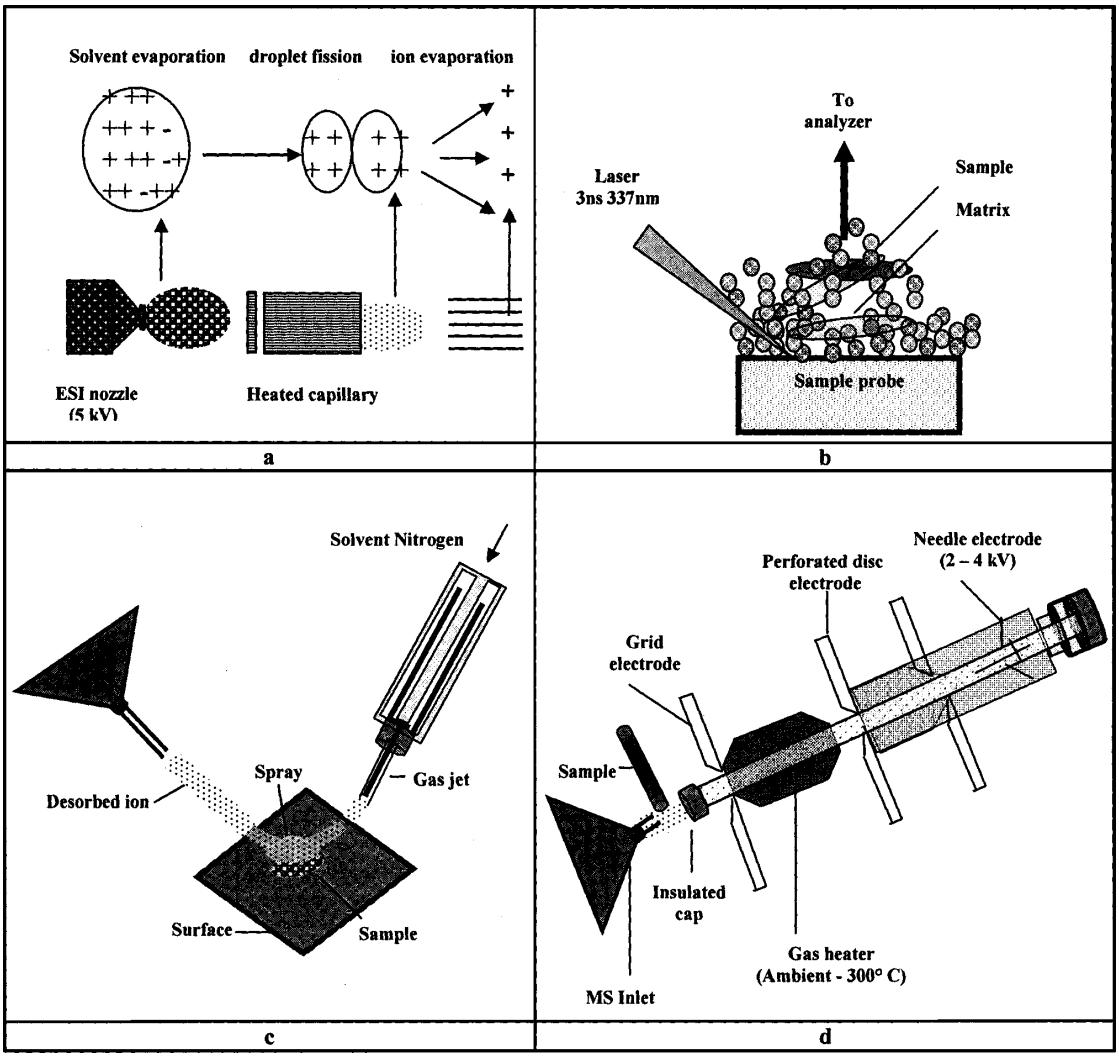

Fig.1. Schematic diagrams of ionization sources: a) electrospray ionization (ESI), b) matrix assisted laser desorption/ ionization (MALDI), c) desorption enhanced laser desorption/ionization (DESI), and d) direct analysis at real time (DART)

ionization potential (IP) of standard solvents, such as methanol and water, which is not ionized by the krypton lamp. Hence, it is much more sensitive than APCI or ESI, with higher signal-to-noise ratio. It is capable of analyzing relatively non-polar compounds[12].

Matrix-Assisted Laser Desorption/Ionization (MALDI) and related techniques

The principle behind the working of MALDI is evaporation of the components from a crystallized sample/matrix mixture with the help of a laser beam (normally a nitrogen laser), which also triggers ionization at 10 mTorr[13] (Figure 1b). It is an appealing technique for biomolecules, such as peptides, proteins and sugars, which turn more fragile and quickly lose structure during ionization by conventional methods. The matrix acts as a receptacle for the analyte and protects it from direct laser ablation. It is similar to ESI in relative softness and with respect to the ions produced, with an added advantage that it is more tolerant to salts and complex mixtures. Common matrix substances employed are crystallized

small aromatic acids, such as sinapinic acid, hydroxycinnamic acid derivatives, and 2,5 dihydrobenzoic acid[14]. These are added to a mixture of highly purified water, an organic solvent (such as acetonitrile, methanol, ethanol or acetone) and trifluoroacetic acid (TFA) in a ratio of 50:50:1. This solution is further mixed with the analyte in proportion of 100:1. The resultant solution is spotted onto a metal plate, designed for the purpose. The solvents vaporize under laser, leaving co-crystallized matrix and the analyte on a MALDI plate to be ionized. MALDI is often modified to work at atmospheric pressure, the technique being called as atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI)[15]. As yet, the disadvantage of AP-MALDI is limited sensitivity and mass range.

Surface-Enhanced Laser Desorption/Ionization (SELDI) is a related technique, where sample is ionized by the use of a light-absorbing matrix, which is added to the spot surface after the purification step[16]. SELDI has a disadvantage of unnecessary background noise at high masses and substantial low resolution, but still it is advantageous for complex biological material (biopsies, microdissected tissue) as it involves no pre-processing or labelling, and uses small amounts of a sample.

Desorption Electrospray Ionization (DESI, Figure 1c), on the other hand, is a new ionisation technique working at an ambient atmosphere, which is capable of extremely low detection limits (fmol for the solid analytes and pmol for the solution analytes)[17]. The method allows high throughput analysis in conjunction with the high speed of analysis, and hence is suited for metabolomics, small spot analysis, and molecular imaging of complex materials, including biological samples. Another laser-based desorption technique is called Desorption/Ionization on Silicon (DIOS), wherein liquid or wet samples are deposited on porous silicon, which is dried and subjected to laser irradiation. It can detect low-mass compounds, as it lacks MALDI matrix background; but is inefficient for higher molecular weight proteins[18].

Direct Analysis in Real Time (DART)

DART is a novel ion source that surmounts many shortcomings of conventional ionization techniques, providing high-resolution and accurate mass measurements of gases, liquids and solids, with further advantage of excluding sample preparation or modification[19, 20]. It is based on atmospheric pressure interactions of long-lived electronic excited state of atom or vibronic excited state of molecules with the sample and atmospheric gases. A gas (He or N2) flows via a chamber where an electrical discharge generates ions, electrons and metastable atoms or molecules (Figure 1d). Charged particles are removed as gases pass through the electrodes. Only the neutral gas molecules, together with metastable species, stay behind. A grid at the exit avoids ion-ion and ion-electron recombination, and acts as the source of electrons by surface penning ionization. By altering the polarity of the electrodes, DART can be operated in a positive as well as negative ion modes. It directly detects drugs and metabolites in unprocessed body fluids, including blood, urine (e.g., uric acid from a drop of urine placed on a glass rod), perspiration and saliva, with no sample preparation of any kind. It can also identify substitute active ingredients that had been added to the counterfeit products. As an example, dihydroartemisinin (an antimalarial drug) and methyl stearate (used in counterfeit antimalarial pharmaceutical preparations), both having the same nominal mass, were detected in a pill without sample preparation of any kind[21].

Inductively Coupled Plasma–Mass Spectrometry (ICP-MS)

ICP-MS is a technique of choice today for determining accurately the limits of heavy metals in pharmaceuticals as well as in analyzing leachables of closures and containers. The ionization in ICP is carried out at a high temperature (usually 8000 K) by self-sustaining electrical discharge, applied by means of a high frequency induction coil, in a flow of argon gas at atmospheric pressure, which forms

a plasma system (containing electrons and charged species)[22]. Samples are introduced into the plasma in nebulized solutions, flowing at rates of 0.5-1 ml/min and are ionized proficiently by the high-temperature plasma. ICP-MS allows multi-element detection and measurement of isotope ratios. Laser ablation ICP-MS is becoming the method of choice for analysis of solid samples due to the advantage of direct solid sampling and its potential of providing micro-scale information.

2. MASS ANALYZERS

Once a sample is ionized, it must be analyzed for its mass value. The beam of ions is focused and directed into a mass analyzer, which separates the ions with respect to their m/z value. The key parameters of a mass analyzer are sensitivity, resolution and mass accuracy. Different types of mass analyzers are described as follows:

Quadrupole

As the name suggests, four parallel rods constitute the electrodes wherein positive and negative terminals of a DC (direct current) source and changeable out-of-phase radio frequency (AC) are applied to each pair of electrodes[23] (Figure 2a). Ions are accelerated between the rods, keeping a stable trajectory in the two planes relative to the applied field. The separation of ions in quadrupole is not directly correlated to their kinetic energies, rather to the relative motion of ions in the dynamic electric field. Ions that are different in the mass unit can be resolved by adjusting the center of the band by modulating AC and DC potentials[24]. It is an extensively employed analyzer for pairing mass instruments with separation techniques, like GC and HPLC. It supersedes other analyzers in terms of ease of quantification.

Ion Trap

It consists of a pair of end electrodes, and a pair of ring electrodes filled with helium at about 1 mTorr. Pulses in electrostatic ion gate guide the entry of ions into a trap (Figure 2b). Combined DC voltages and RF are applied to the electrodes[25]. Initially, field allows all entered ions to oscillate and trap into a potential energy well at the center of the analyzer. Trapped ions are focused into the center of the trap through the use of an oscillating potential called the fundamental RF, applied to the ring electrode. An ion will be trapped in a stable manner, depending upon its m/z value. Ionization period is set to maximize signal and lessen the space-charge effects that result from too many ions in the trap. Collisions with helium reduce the kinetic energy of the ions and serve to rapidly contract trajectories toward the center, enabling trapping of injected ions. The mass spectrum is acquired by scanning the RF and DC fields to destabilize low mass-to-charge ions. These destabilized ions are ejected through a hole in one end cap electrode and strike a detector. Scanning of the fields produces the mass spectrum by ejecting the ions with increasing m/z value from the cell followed by their detection.

Time-of-Flight (TOF)

It exhibits simplicity, ruggedness, unlimited mass range and rapid data acquisition[26]. A conventional TOF assembly consists of a tube where ions of various m/z are separated according to their differential speeds (Figure 2c). It is called linear TOF analyzer. An electronic field accelerates ions with equal kinetic energies that are gained during ionization, into a field-free drift tube of fixed length. The separation of ions is based on their relative velocities within a flight tube, which is directly related to their momentum, a function of mass. Typical flight times are 1-30 ms. However, its main drawback is peak broadening due to variations of ion velocities during flight period for same m/z. The linear TOF instruments are also limited by their intrinsically low resolution. The advent of an electrostatic reflectron containing TOF analyzer proved an important landmark amongst mass analyzers. In reflectron based TOF analyzers, ions are reflected back to the subsequent flight by an electrostatic mirror. This helps in harmonizing the

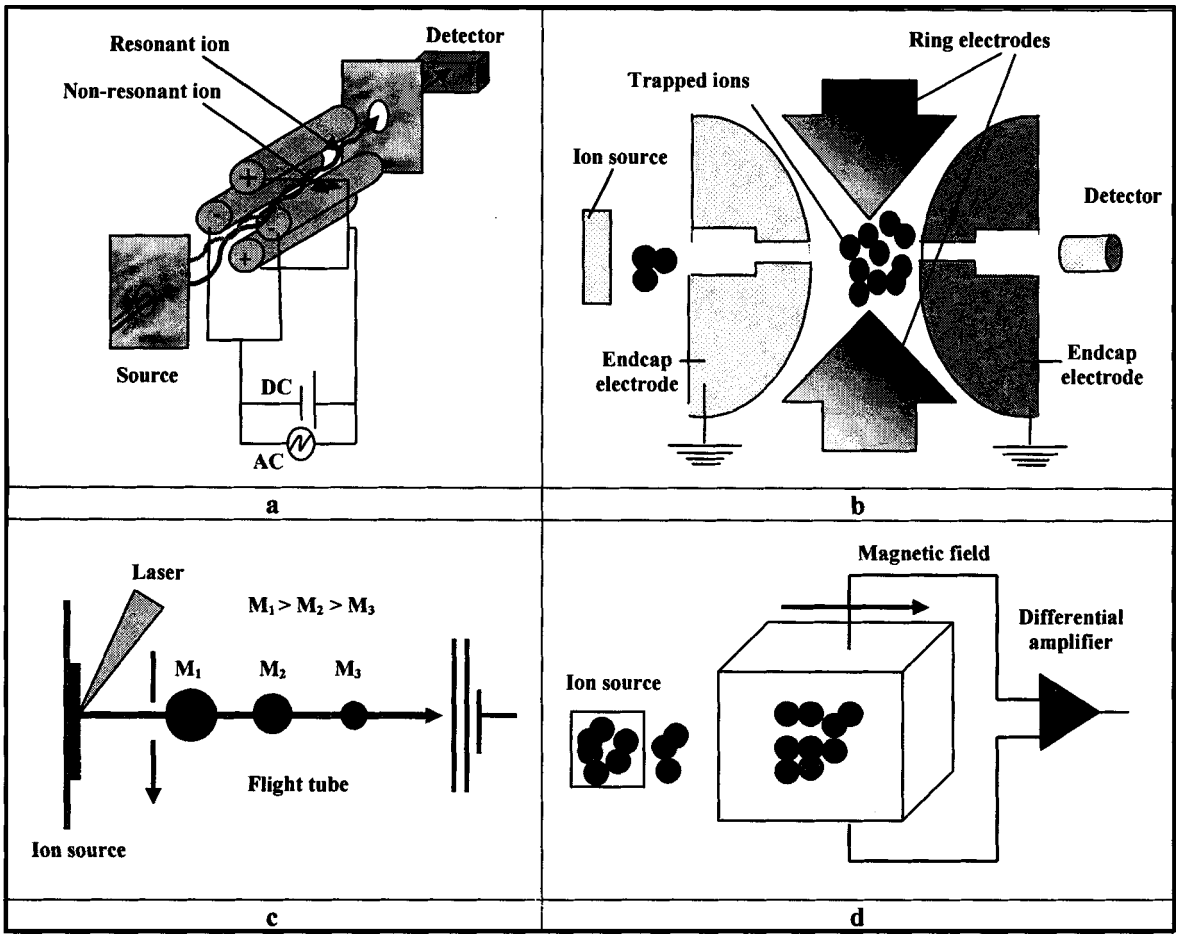

Fig. 2. Different mass analyzers: a) quadrupole, b) ion trap, c) time-of-flight, and d) Fourier transform ion cyclotron

ions with same m/z value and different kinetic energy so as to get same velocities. This leads to the increase in the performance of such instruments[27]. Hence, reflectron based TOF analyzers are gradually replacing conventional linear TOF analyzers.

Fourier Transform-Ion Cyclotron Resonance (FT-ICR)

In spite of their complex mechanics, FT-ICR are the mass analyzers with the highest performance among those currently available[28, 29]. It provides ultra-high mass resolution and mass accuracy, non-destructive detection, high sensitivity and multistage MSn. Ions are drifted into a spatially uniform static magnetic field strength, which causes the motion to become circular in a plane at right angle to the direction of the magnetic field (Figure 2d). The angular frequency inside the trapping cell is inversely proportional to the m/z value. The presence of ions between a pair of detector electrodes in the trapping cell will not produce any measurable signal. It is essential to excite the ions of a given m/z as a consistent package to a larger orbital radius, by applying a radiofrequency (RF) sweep of a few milliseconds across the cell. Fourier transformation allows measurement of all the frequencies simultaneously, each of which excites one particular m/z, thus resulting in a mass spectrum. As the

frequency can be measured more precisely than any other physical property, the technique has an extremely high mass resolution (>106)[30].

Orbitrap

Orbitrap works on the principle of axially electrostatic harmonic orbital trapping. It traps injected ions in an electrostatic field (no magnet, no RF trapping) and detects them with a high resolution, high sensitivity, and high mass accuracy using FT. Due to the high resolving power of the orbitrap, it acts as a second mass analyzer in tandem with linear quadrupole ion trap (LTQ)[31]. The technique has the potential to become a significant mass spectrometry technology, e.g., it has been applied for identification and relative quantitation of positional isomers in phosphoditylcholine (PC) without internal standards[32].

3. DETECTORS

In mass spectrometers, the generated ions are separated, analyzed, digitized and detected by ion detectors. The detectors amplify the subtle response in a way to generate a useful mass spectra[33]. A conventional detector is a Faraday cup, wherein high-speed ions strike the inside of it and release the secondary electrons. The generation of electrons gives rise to a momentary flow of electric current until the electrons are recaptured. It is a simple and robust detector used in circumstances where high sensitivity is not required. Another commonly used detector is electron multiplier, which is composed of a series of dynodes. An incident ion beam knocks out two electrons from the first dynode, which are accelerated towards the second dynode, where each ejects two more electrons. These in turn are accelerated to a third dynode and so on, ultimately reaching, say a tenth dynode, by which time the original two electrons turn into a shower of electrons. Sometimes, dynodes contain scintillator that emits photons, which are detected by using a photo multiplier tube. Since photon multipliers are very sensitive, they are important in studies on metastable ions.

Hyphenation of MS

As the mass spectrometer is a highly selective technique, direct analysis of untreated and raw samples often creates disturbance in characterization/identification or quantification. The composition of matrix may affect response of interest; especially the signal intensity of the monitored species due to other ions concurrently generated in the ion source. Advanced components and high vacuum used in a mass instrument makes it prone to damage and contamination, attributable to undesired components in the samples. These rationales witnessed the need of hyphenation of MS with separation techniques. All hyphenation techniques, those are orthogonal to MS, act as independent components of the system, while MS is dependent component whose output is the function of the former one. As both gas chromatography (GC) and MS work with the gas phase species, their hyphenation is rather unsophisticated, efficient in terms of sample preparation as well as the physical bench space. But it lacks the ability to analyze thermolabile and non-volatile components.

Contrary to it, liquid chromatography (LC) coupled to MS can handle a wide variety of samples[34]. An additional lucrative attribute of LC lies in its super selectivity through the employment of diverse chromatographic supports. The most common is reversed-phase liquid chromatography (RPLC) with hydrocarbon-bonded phases, especially C18-phases, which are most frequently employed today. Mostly, the LC used is high performance liquid chromatography (HPLC) (Figure 3). In more

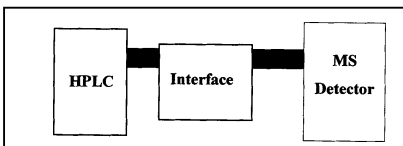

Fig. 3. MS hyphenated with HPLC (LC-MS)

recent times, there has been advent of ultra pressure liquid chromatography (UPLC), which has also been combined with MS[35].

The development of an ionization interface between LC and MS has been the most crucial aspect of hyphenated LC-MS systems. The ideal ionization technique for pairing MS with LC depends upon several factors, including the nature of the analyte, the separation method selected, mass analyzer to be used and various other instrumental parameters. As LC works in highly pressurized atmosphere, it contrasted with the evacuated ionization environment of MS. This led to the specific design of LC and MS interface to avoid entry of solvents in MS system. This was managed by either making the ions to travel through the channels of a heated metallic counter electrode, which guarded the sampling cone orifice by ion desolvatation, or a flow of heated gas that protected the orifice plate and helped in desolvatation. Now-a-days, liquid based separation techniques attached on-line to MS are often employed with ionization at atmospheric pressure (API), which includes APCI, APPI, ESI and some other new techniques. However, most applications of LC coupled to MS use ESI, due to its sensitivity and ability to ionize a large range of analytes. Small polar to medium polar analytes are also readily ionized with this technique, making LC-ESI-MS useful in a variety of pharmaceutical applications.

Versatility of pairing LC with MS is often adapted glamorously by setting the systems for preparative and semi-preparative purpose, so as to be selective in the fraction collection. Unlike UV detector, MS is destructive and very sensitive technique. Therefore, on-line preparative HPLC-MS systems require complex plumbing and precise splitting schemes to split and deliver small amount of effluent to the MS detector;

Table 1: Commercially available tandem mass spectrometers

Types	Commercially available instruments	Features Available with various ionization sources such as ESI,	Manufactures
Hybrid quadrupole	6410 Triple- Quadrupole LC/MS; 6500 Quadrupole-TOF LC/MS	capillary - ESI, nano-ESI, APCI, multimode ESI/APCI, APPI, and MALDI	Agilent Technologies
	Quadrupole-TOF (QSTAR Elite); Triple quadrupole LC/MS (API 5000, 4000, 3000, 2000)	Increased resolving power, mass accuracy at least 2 orders of magnitude	Applied Biosystems / MDS Sciex
	Quadrupolelontrap-TOF (ultra-TOF-Q); BioTOF-Q	Available with combination of ESI and MALDI ion sources	Bruker Daltonics
	LCMS-QIT-TOF	High precursor ion selection, higher mass resolution and accuracy	Shimadzu
Hybrid trap	Q TRAP; 4000 Q TRAP	Selectively full scan, MS3 possible	Applied Biosystems
	LTQ™ Orbitrap; LTQ (2D); LXQ; MAT95XL-TRAP	Trapping instruments are capable of MSn experiments hence of great importance in routine analysis	Thermo Electron
TOF-TOF and hybrid	4800 MALDI TOF/TOF	High sensitivity, ultra-high performance, opti-beam on-axis laser system	Applied Biosystems/ MDS Sciex
	AXIMA-TOF2 high energy CID MALDI-TOF/TOF	Gridless ion path, CID with high collision energy	Shimadzu
	MALDI-TOF/TOF	Higher mass resolution, faster data acquisition, with new laser technique	Bruker Daltonics
	DART AccuTOF; APCI/ESI AccuTOF; GC AccuTOF	High resolution, exact mass measurement, with DART, nondestructive analysis in real time possible	JEOL

while diverting the major fractions to the collection apparatus. Alternatively, preparative LC-MS could also be set off-line by coupling MS independently with preparative HPLC. The preparative LC-MS systems are also employed extensively in drug discovery programs, as these allow high throughput isolation and purification[36].

Other separation techniques used mutually with MS comprise capillary electrophoresis (CE) and electro-chromatography. The hyphenation of LC and ICP-MS can be employed in speciation analyses of samples containing complex heteroatoms. In comparison with ESI, the ionization using ICP-plasma experiences less hindrance caused by buffer additives and/or sample matrix. Therefore, size exclusion, ion exchange and ion pair chromatography can be readily coupled to ICP-MS. One limitation of the ICP-technique is its low tolerance towards organic solvents, such as methanol and acetonitrile, commonly added to the mobile phase in LC, as their vapors cool the plasma, making it unstable or even extinguish it. Various ways of combining more than one orthogonal separation technique with MS are for instance LC-CE-MS, ion exchange chromatography-RPLC-MS and LC-ion mobility-MS. Often, MS is paired with thermogravimetric analyzer, referred to as TG-MS, for analyzing the gases evolved during the operation.

Tandem Mass Spectrometry (Tandem MS)

Tandem mass spectrometry or MS/MS was developed to surge the application of mass instruments to structure elucidation of unknowns and for analysis of complex mixtures, with minimum sample clean up. The technique introduces an additional specificity to recognize a particular component in a mixture. Tandem MS on the whole has two mass spectrometers in a series[37]. Analogous to LC/MS, a sample is sorted in MS1, which is then broken into fragments in the collision cell, whereafter these fragments are sorted in the MS2. In other words, the first mass spectrometer (MS1) selects ions of a particular m/z value, called the precursor or parent ion, which is generated from primary fragmentation. The latter afterwards passes into the fragmentation region or collision cell. The daughter ions are then analysed in the second spectrometer (MS2).

The collision activation in collision cell can be of either high or low energy, selected according to the analyzer used and is controlled by electrically insulated cell held at a high potential to retard the ion beam, which is reaccelerated on exit[38]. The commonly used dissociation method is collision-induced dissociation (CID), in which the parent ion collides with a neutral gas (He, Ar, N_2, etc.) at high pressure, converting a part of its kinetic energy into the internal energy, giving rise to daughter ions. Other collision techniques are surface-induced dissociation[39] (SID) involving collision with a self-assembled monolayer surface, and photodissociation, where ions are dissociated by absorption of photons. Of late, more devices have emerged into the category, viz. sustained off-resonance irradiation collision-induced dissociation (SORI-CID), infrared multiphoton dissociation (IRMPD) and blackbody infrared radiative dissociation (BIRD). The latter ones are mainly implemented in trapping instruments.

Generally, tandem mass spectrometers are operated in three different modes (Figure 4). The first is the product ion-scanning mode; where products ions are scanned for the predetermined parent ion by making MS1 independent, while MS2 constitutes the dependent variable[40]. The second is the precursor ion-scanning mode, which is almost reverse to the first, where MS2 is fixed for the predetermined product ion, while scanning in MS1 for parent ions becomes a dependent variable. The third mode is the neutral-loss scanning mode, where neutral component of interest, which is gained or lost between MS1 and MS2, is set as an independent variable and accordingly both MS1 and MS2 are scanned. All

these modes are set within the instrument as per the necessity.

Other elegant aspect of tandem systems is the reaction monitoring, where parameters of MS1 and MS2 are set in such a way, to allow choice of particular parent and product ions. Two types of experiments are employed, the simpler case being the single reaction monitoring (SRM), where there is a single product ion corresponding to a single parent ion. Other one is multiple reaction monitoring (MRM), where one or more precursor ions are monitored for two or more product ions, which can be subjected to necessary permutations to yield the most relevant information. In ion trap and FT-ICR based equipment, it is feasible to repeat this process to n number of stages, leading to MSn fragmentations[41].

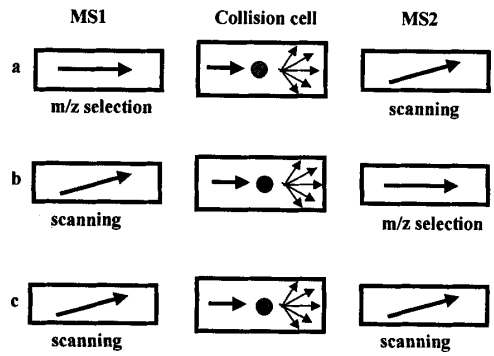

Fig. 4. Different modes of scanning in tandem mass spectrometer: a. product ion scan, b. parent ion scan, c. neutral loss scan

Tandem instruments are of two types; tandem-in-time and tandem-in-space[41]. In tandem-in-time type, different phases of MS/MS are carried out in the same analyser, but separated in time. The trapping instruments fall in this category. This even facilitates achievement of MSn experiments. However, the trapping instruments fail to provide scanning of the parent ion as well as the neutral ion. In case of tandem-in-space, each stage of MS/MS needs distinct analyser and normally beam type analyzers suit this experiment and all types of scanning can be performed with these analysers. A typical example is a triple quadrupole (QqQ), where three quadrupoles exist in linear series. The first quadrupole is a usual analyser, while the second and the third act as CID and ion trap, respectively. The QqQ can be coupled either with TOF or the ion trap to give a variety of tandem mass spectrometers, and these are extensively used in industry today.

PHARMACEUTICAL APPLICATIONS

DRUG CANDIDATE SYNTHESIS AND SCREENING

Modern drug discovery practice is heavily dependent on the synthesis of combinatorial libraries. As the preparation of large libraries for lead discovery became routine, emphasis is going to be placed on analytical techniques that focus mainly on throughput and quality. Analytical and preparative RPLC in conjunction with MS offers such a potential[42, 43]. As emph asis increases on speed, modern trap and TOF instruments can be employed to measure many mass spectra per second. On the other hand, attraction towards FT-ICR is increasing in the pharmaceutical industry owing to its capability to deliver more information per measurement. The instrument has undergone rapid development and is now applied in many fields. FT-ICR has contributed to the dereplication and chemical fingerprinting of natural products, such as in bioactive marine macroorganisms and elemental composition determination of the natural products, such as antibiotics[44]. A variety of approaches, based on various combinations of ionization and mass analysis techniques, are integral even to proteomics[45].

METABOLITES IDENTIFICATION AND BIOANALYSIS

Fast and accurate methods in drug metabolism and pharmacokinetic (DMPK) studies are highly demanding in drug discovery programs. The tandem LC-MS systems allow a chemically diverse range

of drug candidates to be analyzed quickly[46]. The LC-MS-based in vivo pharmacokinetic screening approaches allow analysis of large number of samples, thereby reducing the time in which results can be obtained. In a conventional set-up, single auto-sampler/HPLC column, and MS systems are used and samples are injected one at a time. The strategies used to amplify throughput include fast chromatography, automated data processing, and pooling strategies (cassette dosing, pooling after individual dosing, simple sample screens, etc). Two auto samplers and columns, in parallel with a single triple quadruple mass spectrometer, reduce HPLC column equilibration time and common holdups associated with sample loading. The LC-MS and LC-MS/MS techniques are also very useful in metabolite identification, as they have the ability to predict as well as detect the metabolites in complex samples of urine, bile, and plasma[47]. The metabolite prediction is based on the fact that metabolites are produced from select in vivo reactions. The software then imposes the predicted metabolites upon the resolved ones and carry out detection based on the fragmentation overlap. Also, in general, metabolites preserve most of the core structure of the parent drug, and hence show similar fragmentation pattern, generating mass spectra that designates major substructures.

IMPURITY IDENTIFICATION AND PROFILING

Impurities in drug substances are synthetic by-products, starting materials, intermediates, etc., while in case of drug products these constitute degradation products formed on storage. Toxic impurities, regardless of source, are of particular concern during safety evaluation, process research activities and during dosage form development, because of the rigorous regulatory requirements outlined by various international agencies, including ICH[48]. The mass fingerprinting techniques, like LC-MS/TOF, LC-MSn or the recently introduced LC-MSn-TOF have proven to be very useful tools for on-line characterization and structure elucidation of impurities in trace concentrations. Alternately, mass based preparative instruments are employed for specific isolation[49]. When a new impurity is encountered, retention time, molecular weight information and sub-structural fragmentation pattern obtained using MSn and/or TOF helps in rapid identification[50, 51]. CE-MS has also been implemented in the impurity profiling of pharmaceutical products[52].

STABILITY STUDIES OF DRUG SUBSTANCES AND DRUG FORMULATIONS

During the development phase, drug substances as well as drug formulations are subjected to stress testing under a variety of stress conditions, such as temperature, humidity, acidity, basicity, oxidation, light, etc. The same facilitates validated analytical method development and provides extrapolative information for upcoming formulation and packaging studies. For these studies, practical approaches to attain structural elucidation of degradation products using modern LC-MS or LC-MS/MS techniques and information obtained form them, such as retention time, molecular weight and fragmentation pattern, has gained paramount importance. The strategy for identification of degradation products during early development requires fast and sensitive LC-MS analytical methods[53]. Consequently, a structure database can be built from outcomes, which is employed to distinguish unstable structures within the drug structure as well as for the rapid identification of degradation products generated during these studies[54]. These techniques are also extended later during the analyses of long term as well as accelerated st1udy stability samples to obtain useful and relevant information.

CONCLUSIONS AND FUTURE PROSPECTS

The inherent analytical advantages of mass spectrometry, including speed and sensitivity, combined with recent advances promise to make the technique a mainstay with respect to variety of pharmaceutical

applications. The recently developed ionization techniques, such as DART and DESI, and analyzers, such as orbitrap, allow any type of component to be studied by MS. In addition, MS/MS, MSn and MS/TOF add the capability for structural analysis of compounds that are present at low level and/or are present in complex mixtures.

The sensitivity and high throughput capability of MS should make it a powerful tool for the early stages of drug discovery, including combinatorial chemistry and DMPK studies. The same is the case even with routine analysis, including impurity profiling, stability testing and quality control activity. The gentle nature of relatively new ionization techniques and the structural analysis capability of MS have the promise to extend mass spectrometry towards more new areas[55].

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MOLECULAR MECHANISMS OF APPETITE SUPPRESSION, OBESITY REGULATION AND ABDOMINAL FAT GENE EXPRESSION FOLLOWING SUPPLEMENTATION OF A NOVEL (–) - HYDROXYCITRIC ACID EXTRACT

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Obesity is a major challenge to the health professionals. Currently, the estimated mortality from obesity-related diseases in the USA is approximately 300,000 annually and growing. WHO records show that globally 315 million suffer from obesity. In near future, obesity-related mortality is expected to exceed that of smoking.

The dried fruit rind of Garcinia cambogia (family Guttiferae), also known as Malabar Tamarind, is a unique source of (-)-hydroxycitric acid (HCA), which exhibits a distinct sour taste and has been used for centuries in Southeastern Asia to make meals more filling. HCA has been demonstrated to inhibit ATP-citrate lyase (a building block for fat synthesis), and effective in weight management in experimental animals and in humans. A significant amount of research has been conducted in our laboratories on a novel, highly water soluble, calcium-potassium salt of 60% HCA extract from Garcinia cambogia (HCA-SX, commercially known as Super CitriMax). A broad spectrum of studies including acute oral and dermal toxicity, primary dermal and eye irritation, 90-day chronic oral toxicity, Ames' bacterical reverse mutation assay and mouse lymphoma tests, demonstrated the safety of HCA-SX. HCA-SX was shown bioavailable in the human plasma by GC-MS. HCA-SX also acts as a mild serotonin receptor reuptake inhibitor and enhances the availability of serotonin in the brain tissues. HCA-SX feeding marginally increased five neurotransmitters including serotonin, dopamine, DOPA-C, 5-HIAA and HVA in the brain tissue, which might explain its appetite suppressive behavior.

A recent clinical study in 90 obese subjects exhibited that HCA-SX reduces body weight, BMI, serum leptin (a biomarker of obesity regulatory gene), LDL, triglycerides and total cholesterol, and enhances the excretion of urinary fat metabolites (fat oxidation) and serum serotonin level. To understand the mechanistic aspects behind the obesity regulatory role of HCA-SX, the effects of low-dose oral HCA-SX was investigated on the body weight and abdominal fat transcriptome in rats. Sprague-Dawley rats were fed either control or HCA-SX for 8 weeks. Total RNA was extracted from abdominal fat and microarray Genechip analysis was conducted using Affymetrix Microarray Suite 5.0. Results were validated on selected genes by conducting Real Time PCR. HCA-SX restricted body weight gain in rats and lowered abdominal fat leptin expression. High-density microarray analysis of 9960 genes and ESTs present in the fat tissue identified a small set of specific genes sensitive to dietary HCA-SX and weight loss. Mitochondrial/nuclear proteins necessary for fundamental support of the tissue were not affected by HCA-SX, which further reconfirmed its safety. Functional characterization of HCA-SX sensitive genes revealed that up-regulation of genes encoding serotonin receptors represents a distinct effect of HCA-SX on appetite suppression.

Taken together, these results reconfirm the ability of HCA-SX in weight management by modulating a set of key functional genes.

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MEDICINAL HERBS-SPILANTHES SPECIES: A REVIEW

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ABSTRACT
Toothache plants (various *Spilanthes* spp.) are widely distributed in tropics and subtropics. They occur as wild and cultivated too. These have been used popularly by traditional physicians for treatment of various ailments. Few *Spilanthes* spp. have been chemically investigated for presence of their active constituents. Different extracts and purified compounds have been subjected for pharmacological experiments. This paper presents a review of the work done so far on various *Spilanthes* spp.

Keywords. *Spilanthes* spp., Asteraceae, Toothache Plants, N- isobutyl amides, Spilanthol.

INTRODUCTION
Toothache plants are annual herbs, or short-lived perennials, approximately half meter tall with prostrate or ascending branched cylindrical hairy stems and simple ovate opposite leaves with stipules. They belong to family Asteraceae, the tribe Helianthae, and the sub-tribe Ecliptinae. They have characteristic flower heads, which distinguishes individual species. *Spilanthes acmella* L. var. *paniculata* has yellow flower head, while *Spilanthes acmella* has got violet flowers[1]. From Andhra Pradesh, *Spilanthes paniculata* ex DC., *S. radicans* Jacq. and *S. uliginosa* Sw. are reported[2]. Identification of *Spilanthes* spp. has been discussed from time to time[2-4]. It originated in Africa and South American tropics[5]. The herb is widely distributed in tropics and sub-tropics including tropical America, North Australia, Africa, Malaya, Borneo, India, Sri Lanka etc.[3,6,7] About sixty species of *Spilanthes* have been reported from various parts of the world including India[3]. The genus occurs widely in damp pastures, at swamp margins, on rocks near the sea and as a weed of road-sides and cultivations[8]. In India, these are reported from south India, Chhatisgarh and Jharkhand. In Sri Lanka, *Spilanthes acmella* Murr. occurs commonly in moist places up to 1800 m altitude[7]. Few species of *Spilanthes* including different varieties such as *Spilanthes acmella* L., *S. acmella* L. var. *oleracea* Clarke, S. *acmella* Murr., *S. acmella* var. *calva, S. paniculata* (DC) Jansen, *S. oleracea* Jacq., *S. alba.*, *S. ocymifolia* etc. have been popularly called as toothache plant1[6-10]. Other common names include Eyeball Plant, Spot Plant, Para Cress (after the Brazilian province), Brazil Cress, Alphabet Plant (from the Flemish ABC Kruid), and Australian Cress[11]. In Hindi, it is known as Akarkara[4].

CULTIVATION
Spilanthes is reported as a perennial in the tropics and sub-tropics, but it may grow as an annual in temperate regions. The seed germinates vigorously in about 12 days under greenhouse conditions (21-32°C). Damp and cool conditions are held responsible for rotting of seeds. The plants should be started indoors or in the greenhouse, so that the seedlings become well established at the time of transplanting to the garden. Transplantation should be done in the evening. *Spilanthes* is a heavy feeder, preferring rich soils and an occasional side dressing of organic compost. Stem cuttings may also be used for propagation of the plant. The leaf and buds may be harvested on an ongoing basis, as often as the plant can afford[12].

Shoot and root length, biomass, basal stem, leaf area, overall size, number of inflorescences and flowers and seed production were all enhanced in *Spilanthes calva* in the presence of the fungus

Piriformospora indica, a plant growth-promoting root endophyte[13]. Increase in the antifungal potential of *S. calva* was observed due to inoculation of endophyte *P. indica*. The antifungal activity of the plant was enhanced due to the increase in spilanthol content after inoculation of *P. indica*[14].

ACTIVE CONSTITUENTS

Phytochemically, flowers of *Spilanthes acmella* are reported to contain amino acids[15,16], alkaloids[16], *N*-isobutylamides (spilanthol, undeca–*2E,7Z,9E*–trienoic acid isobutylamide and undeca–*2E*–en-8,10-diyonic acid isobutylomide)[8]. A number of other *N*-isobutylamides such as *2E*–*N*-(2-methylbutyl) –2–undecene–8,10–diynamide; *2E,7Z*–*N*-isobutyl–2,7-tridecadiene–10,12–diynamide and *7Z*-*N*-isobutyl-7-tridecene-10,12-diynamide from *Spilanthes acmella* have been reported[1]. *N*-isobutylamides have also been reported from other species such as *Spilanthes alba*[17] and *Spilanthes oleracea*[18]. *N*-2-Phenylethylcinnamamide was isolated from *Spilanthes ocymifolia*[19]. Myricyl alcohol, α- and β-amyrins, β-sitosterol, stigmasterol and other compounds have been isolated from the air dried whole plant of *Spilanthes acmella*[20,21]. Essential oil containing the major constituents as limonene (23.6%), β-caryophyllene (20.9%) (Z)-β-ocimine (14%), germacrene D (10.8%) and myrcene (9.5)% has been obtained from the flower heads of *Spilanthes acmella*[22]. Presence of a mixture of C22 to C35 normal hydrocarbons in the flower heads of *Spilanthes acmella*[23] have been reported. Flavonoid glycoside was also isolated from *Spilanthes Calva*, which was characterized as tetrahydroxdihydrochalcone 3'-0-glucoside, the compound possessed hypoglycemic activity in mice where diabetes was induced by streptozotocin[24]. From the aerial parts of Spilanthes leiocarpa, a new compound eudesmanolide was reported[25]. Secondary volatile metabolites including sesquiterpenes (α- and ß-bisabolenes, caryophyllene and cadinenes), nitrogenated (N-(isobutyl)-*2E,6Z,8E*-decatrienamide; *N*-(2-methylbutyl)-*2E,6Z,8E*-decatrienamide; decatrienamide; N-(isobutyl)-6Z,83-decadienamide and *N*-(2-phenelethyl)*2-E,6Z,-8E*-decatrienamide) and oxygenated compounds have isolated by simultaneous distillation-solvent extraction (SDE) and supercritical (CO_2) extraction (SFE) from flowers, leaves and stems of *Spilanthes americana* (Mutis) Hieron. SFE extracts from stems were found rich (>40%) in sesquiterpenes, while those from leaves and flowers were abundant in nitrogenated (43 and 27%) and oxygenated (36 and 23%) compounds[26].

TRADITIONAL USES

The flowers and leaves of *Spilanthes acmella* L. and *S. acmella* L. var. *oleracea* Clarke have a pungent taste accompanied by tingling and numbness and have been used as folk medicine for stammering, toothache, stomatitis, and throat complaints[1]. In India, roots, leaves and flower heads of Akarkara are used as medicine. The flower heads of *S. acmella* var. *calva* and *S. paniculata* (syn. *S. acmella*) are the most pungent part and Indian traditional healers use it in dental and gum care. It is one of major ingredients in popular herbal tooth powders and paste[3]. In Sri Lanka, the flowers are chewed or used in the form of a tincture for toothache and to stimulate flow of saliva (sialagogue)[7]. In case of degenerative gum disease, *Spilanthes* mouthwash regimen (a little *Spilanthes* extract into a glass of clean water, using this as a swish after tooth brushing for several months) stimulated blood circulation in the oral mucosa, in conjunction with a rapid flushing of all the tooth and gum interstices by the freely flowing saliva, cured the disease[12].

Sri Lankan traditional physicians especially in the Uva province claimed that the cold infusion of the flowers of *Spilanthes acmella* has potent diuretic activity and the ability to dissolve urinary calculi[7].

Spilanthes is one of the few herbs, which exhibits antimalarial properties as well. Spilanthol is found effective at extremely low concentrations against blood parasites, and indeed is a poison to most invertebrates while remaining harmless to warm-blooded creatures. This is the explanation for its utility against blood parasites, specifically malarial spirochetes, either as a prophylactic or as a treatment for malarial paroxysms[12]. It is antifungal, antiviral and immunostimulating[27]. The herb also possesses a strong antibacterial activity. Studies have shown strong in-vitro activity of *Spilanthes* extracts against common pathogens such as *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella gallinarum* and *Staphylococcus albus*. *Spilanthes* also inhibits the yeast/fungal organism such as *Candida albicans*, which is responsible for the nearly epidemic condition known as candidiasis[12]. *S. acmella* also possessed excellent anti-microbial activities against red halophillic cocci from salt cured fish[28]. Researchers have shown preliminary antimicrobial activity in the crude extract from roots and flower heads of *S. mauritiana*[29,30]. In Amazon, *Spilanthes acmella* has been used for the treatment of tuberculosis by laymen[31].

A study was done on dried *Spilanthes* leaf from Rwanda showing active immunomodulating activity, specifically increased production of mononuclear leukocytes[12]. In case of earache and ear infections, the immune enhancing activity of the Echinacea was enhanced by follow up with anti-bacterial influence of the *Spilanthes* tincture to cure the problem[12].

In the Cameroons the plant is used as a snakebite remedy and in the treatment of articular rheumatism[32]. It is also recommended for paralysis of the tongue and stammer and sore mouth in children. Flowers are used in Sri Lanka against itching and psoriasis.

MODERN USES

The larvicidal potential of *Spilanthes acmella* against *Culex quinquefasciatus* has been shown by Pitasawat et al. (1998)[33]. Ether extract of the fresh flowering tops is lethal to *Anopheles* mosquito larvae and American cockroach[34,35]. *S. mauritiana* contains alkaloids that were found to be able to control *Aedes aegypti* in Kenya[36].

Highest dose (1500mg/kg) of cold water extract (CWE) of *Spilanthes acmella* flowers tested, possessed strong diuretic activity when given orally to rats in a single dose. The CWE was well tolerated with an encouraging safety profile even following subchronic administration as judged by absence of mortality, overt signs of toxicity, stress, behavioural abnormalities and increased levels of serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, creatinine and urea. The CWE also claimed as Reno protective (in terms of serum urea levels) in rats[37]. The diuretics induced by the CWE of *Spilanthes acmella* flowers was reported strong with an intensity similar to that of furosemide and accompanied by marked increases in both urinary Na^+ and K^+ levels, this finding was similar to Loop diuretic. This type of diuretics also cause acidification of urine[38-40]. The CWE was also unlikely to be acting as thiazide diuretics[38,40]. The diuretic activity of CWE might be attributed to its alkaloids[37].

Hexanic extracts of *Spilanthes acmella* plants in rats is reported to induce full tonic-clonic convulsions accompanied by typical electrographic seizures in the EEG[44].

Spilanthes calva extract at 2 mg/plate inhibited tobacco-induced mutagenesis (evaluated by Ames *Salmonella*/Microsome assay) by 86.4%[42].

USES AS FOOD

The leaves of *Spilanthes acmella* have been used as a salad ingredient. In Central and South America, *Spilanthes oleracea* is widely used as food ingredient and as medicine. Para Cress (also known as Jambú in Brazil) is used in salads and with sauces, cooked in foods, and used as spice. Its alkaloid spilanthol and a range of alkylamides especially isobutylamides are similar to those found in *Echinacea angustifolia* and E. purpurea[6]. Additions of a few leaves to salads, sandwiches, steamed vegies, and stirfries were suggested to control the symptoms of various types of infections and stimulation of immunity in general[9].

PLANT TISSUE CULTURE STUDIES

Multiple shoot regeneration and transplantation of micropropagated plantlets of *Spilanthes acmella* Murr., were achieved[43]. In *Spilanthes acmella* (L) Murray, multiple shoots were regenerated in MS medium using different concentrations of BAP and KN and different combinations of BAP with IAA, NAA and IBA. Organogenesis was maximum in callus cultured on MS medium supplemented with BAP (2.0 mg/l) and IAA (1.0 mg/l)[44]. MS medium supplemented with 0.5 mg/l BA was sufficient for the proliferation of rooted multiple shoots of *S. acmella* L.[45]. Authors of this review paper have successfully achieved micropropagation and hairy root culture of *Spilanthes paniculata* (DC.) Jansen (unpublished work, in the process of publication). *Spilanthes mauritiana* DC., an East African medicinal herb containing pharmaceutically promising secondary metabolites, had successfully been raised in vitro through axillary bud cultures[46].

CONCLUSION

In conclusion, it could be stated that *Spilanthes* spp. are wonder drugs possessing antibacterial, antifungal, antiviral and antiprotozoal activities. In addition to these, they also possess sialagogue, immunostimulating and diuretic activities.

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EXPLORING PHYTOMEDICINES FROM BIOLOGICAL DIVERSITY

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ABSTRACT

Plants and herbal products have been used as major component of therapeutic agents and sources of chemical diversity in drug discovery programs. In recent years, renewed interest has been shown in the use of medicinal plants and traditional medicine as Phytomedicines and Nutraceuticals. Pushed by the accelerating search for new active ingredients, pharmaceutical companies are actively screening medicinal plants for potential new active substances. The rationalization of the new multidrug and multitarget concept of therapy in classical medicine will have also great implication on the future basic research in Phytomedicines and an evidence based Phytotherapy. It requires a concerted cooperation between phytochemists, molecular biologists, pharmacologists and clinicians with the aim to use modern high-tech methods for the standardization of phytopreparation, to integrate new molecular biological assays into the screening of plant extracts and plant constituents and to increase the efforts into the efficacy proof of phytopreparations by controlled clinical trials which should be paralleled or followed pharmacokinetic and bioavailability studies.

INTRODUCTION

Biological Diversity Act 2002 is a part of the Indian attempt to make some progress and to operationalize the two important provisions of the Convention on Biological Diversity. Biological diversity responds to a number of new, emerging concerns including, the result of new developments in technology, in particular, biotechnology and information technology, and the ongoing degradation of the environment, inevitably accompanied by an erosion of biological diversity. However, in the recent past less emphasis has been placed on higher plants as compared to other natural product sources, especially microorganisms. The important, if not predominant, consideration fostering this lack of interest in higher plant-derived leads has to do with the issue of sustainable and economic supplies of the bulk drug to meet the needs of the drug development process and ultimately the clinical use of the drug. A brief overview of alternative strategies to resolve supply issues and their relative strengths and weaknesses is presented. This has prompted the development of two often conflicting international agreements, the Trade Related Intellectual Property Rights provisions (TRIPS) of GATT and the Convention on Biological Diversity (CBD). The latter has two notable stipulations. One is the sovereign right of countries of origin over their genetic and biological diversity resources. The other is the acceptance of the need to share benefits flowing from commercial utilization of biological diversity resources with holders of traditional knowledge and practices of conservation and sustainable utilization of these resources. There is as yet no proper resolution at the international level of how these will be implemented in view of the fact that the normal Intellectual Property Rights and TRIPS provisions do not stipulate any sharing of benefits for holders of knowledge in public domain, nor the sovereign right of countries of origin over their genetic and biological diversity resources. TRIPS even include intellectual property rights over microorganisms and plant varieties.

INPUT INTO THE RESEARCH AND DEVELOPMENT THROUGH BIOLOGICAL DIVERSITY

The major focus of the research carried out on the value of genetic resources has been on their use in the pharmaceutical and agricultural industries, which use genetic diversity as a source of information

in their development of new products. Economists have long analyzed the research and development process as one of information utilization, application and diffusion. The concept of research and development is usually presented as a production process itself dependent upon a stock of "information" for its generation of useful innovations .The information that genetic diversity represents may be brought into commercial use in one of three ways: the information contained in a genotype may be transferred to the desired end directly (i.e., through transferring genetic material), traditionally through breeding and hybridization between closely related organisms, and more recently through novel technologies of gene transfer; the information can be exploited directly through the expressed phenotype of the organism, so that new organisms are brought directly into commercial production; and the information may be used to develop new products without translocating the biological material. Pharmaceutical industries most often pursue the third of these means (making use of observed strategies in biological material), while agricultural industries most often pursue the first[1]. For example, pharmaceutical companies screen diverse life forms in order to ascertain the presence of chemicals with biological activity (e.g. "alkaloids" in plants). If this information is identified as having some useful potential, then the pharmaceutical industry will, within a laboratory environment, usually focus on the synthesis of that activity from basic chemical constituents. On the other hand, agricultural and plant-breeding companies have, in the past, operated almost exclusively through the identification of useful traits within closely related organisms and the selective breeding for the transport of those genotypes into a particularly useful strain. Although the two industries are pursuing the same basic object (i.e. the incorporation of successful biological strategies into the human economic system), they us contrasting techniques to effect this endeavor. Both industries make limited use of the second of these means the introduction of new organisms directly into the production process. This is also employed with some frequency by the horticultural industry.

HERBAL DRUG DEVELOPMENT

Ethno pharmacologic leads have resulted in the introduction of new single molecule drugs but have a greater role to play if crude extracts are accepted for clinical use in the West. Recent years have witnessed a renewed interest in plants as pharmaceuticals in the Western world. This interest is channeled into the discovery of new biologically-active molecules by the pharmaceutical industry and into the adoption of crude extracts of plants for self-medication by the general public. In both of these areas some attention is being paid to the investigation and use of ethno pharmacology, the traditional use of plants for medicinal purposes by particular cultural groups[2]. The problems confronting such usage are discussed. Considerable benefits for developing countries are possible when the local medicinal plants are subjected to scientific methods of validation of traditional use and quality control[3]. This approach has met with success in some parts of the world but is not always appreciated by national governments and international agencies. Related areas of concern such as conservation of ecology and culture must be integrated with any such program. Plants used in traditional medicine therefore have an important role to play in the maintenance of health in all parts of the world and in the introduction of new treatments[4].

About 74% of these were discovered by chemists who were attempting to identify the chemical substances in the plants that were responsible for their medical uses by humans. There are 119 drugs of known structure that are still extracted from higher plants and used globally in allopathic medicine. These 119 plant-derived drugs are produced commercially from less than 90 species of higher plants. Since there are at least 250,000 species of higher plants on earth, it is logical to presume that many

more useful drugs will be found in the plant kingdom if the search for these entities is carried out in a logical and systematic manner[5]. The first and most important stage in a drug development programme using plants as the starting material should be the collection and analysis of information on the use(s) of the plant(s) by various indigenous cultures. Ethno botany, ethno medicine, folk medicine and traditional medicine can provide information that is useful as a 'pre-screen' to select plants for experimental pharmacological studies. Examples are given to illustrate how data from ethno medicine can be analysed with the aim of selecting a reasonable number of plants to be tested in bioassay systems that are believed to predict the action of these drugs in humans. The ultimate goal of ethno pharmacology should be to identify drugs to alleviate human illness via a thorough analysis of plants alleged to be useful in human cultures throughout the world. Problems and prospects involved in attaining this goal are discussed.

WHY PLANTS?

Using plants to produce pharmaceutical proteins presents several clear advantages. Chemicals derived from higher plants have played a central role in the history of mankind. Efforts to develop new, clinically effective pharmaceutical agents have relied primarily on one of five approaches, most of which utilize existing agents in some manner as follows:

- Derivatization of existing agents
- Synthesis of additional analogs of existing agents
- Use of combination therapy of existing agents with other drugs
- Improvement of delivery of existing agents to the target site
- Discovery of new prototype pharmaceutical agents

While approaches 1 - 4 are important and need to be continued in that they seek to utilize existing agents and information in the most effective manner, there is an urgent need for the development of totally new, prototype agents which do not share the same toxicities, cross resistance or mechanism of action as existing agents. Natural products have, in the past, provided a rich source of such prototype bioactive compounds and it is essential that the search for new drugs pursue this route[6]. The major advantage of this approach is the likelihood of identifying new prototype drugs with quite different chemical structures and mechanisms of action and hence, lowers likelihood of similar toxicity and cross resistance.

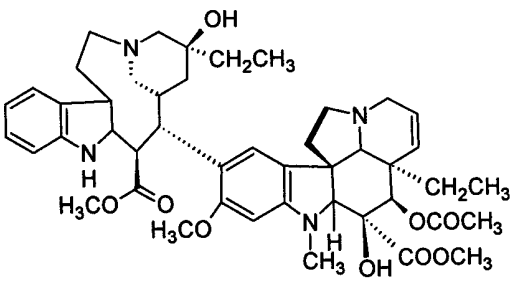
The fundamental element of a drug discovery program is the bioassay(s) utilized to detect preparations with the desired biological activity. The bioassay protocol selected for the discovery of new prototype drugs must meet a variety of criteria. In addition to the expected criteria of ease of operation and low to moderate cost, the assay must show specificity and sensitivity. An important requirement of the assay is its ability to serve as a guide during the bioassay-directed phase of purification of agents showing activity. This is especially so in the discovery of substances from natural sources since these materials are likely to be in very low concentration in very complex mixtures. Only combination of procedures meets these demanding criteria to serve as primary screens for biological activity[7]. Other important program elements must be coupled to the appropriate bioassay. The probability of selection and procurement of novel sources of potential preparations must be demonstrated as well as evidence of competency to accomplish bioassay-directed purification and structure elucidation. Initially detected

activity must be confirmed in suitable secondary and tertiary assays, which will help to define the potential of the substance for clinical utility.

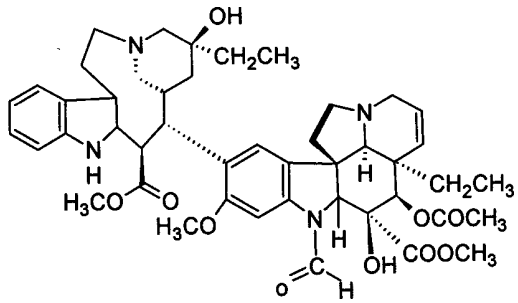
PHARMACEUTICAL POTENTIALS FROM PLANTS

Several factors have contributed to the revival of interest in plant-derived products as follows:

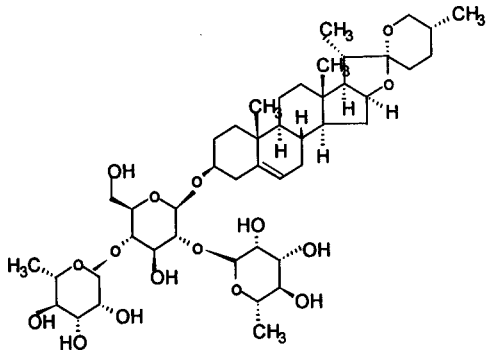
There is an undisputed clinical efficacy of several natural product anticancer drugs. The early discovery of vincristine / vinblastine (I-II) from Catharanthus roseus (the Madagasar periwinkle) was followed by other agents including the aryl lignam etoposide derived from Podophyllum species (mayapple), 'and the taxoids' from Taxus brevifolia (Pacific yew) and T. baccata (European yew). Compounds with less direct therapeutic potential may offer new molecular templates for the design of more effective drugs e.g. the development of atracurium and related muscle relaxants from the alkaloids of curare, the South American dart poison obtained from Chondrodendron tomentosum[8].



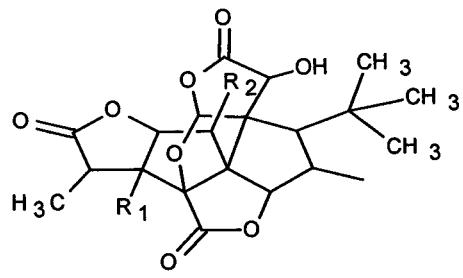
I. vinblastine (Catharanthus rosesus)



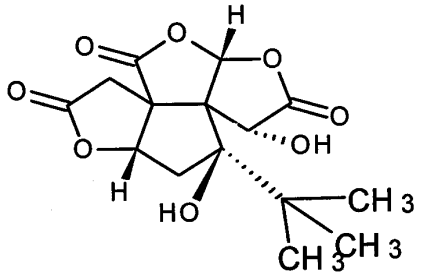
II. vincristine (Catharanthus rosesus)



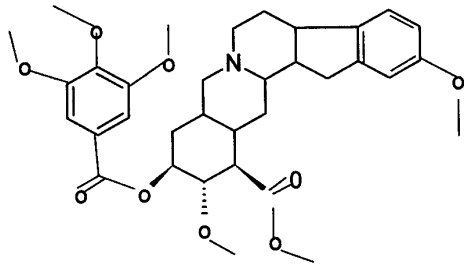
III. Dioscin (Dioscorea villosa)



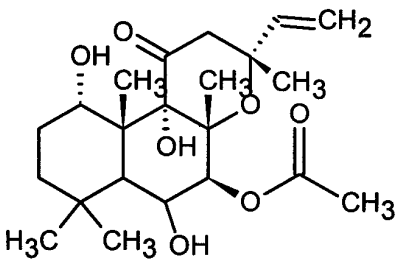
IV. Ginkgolides (Ginkgo biloba)



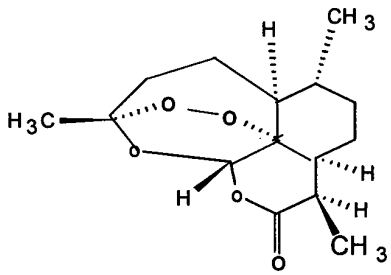
V. Bilobalide (Ginkgo biloba)



VI. Resperine (Rauwolfia serpentina)



VI. Forskolin (Coleus forskholii)



VIII. Arteminisin (Artemisia annua)

Natural products can offer an alternative to established therapy because they act at a different stage in the disease, and be useful in combination therapy. The search for synthetic molecules active against human immunodeficiency virus (HIV) has resulted largely in reverse transcriptase inhibitors, but investigations into plant extracts have produced a wide range of chemical compounds with various modes of action that result in viral non-proliferation.

Plants have proved invaluable as inexpensive sources of "feedstock" molecules that can be readily transformed into drugs. Thus, development of the steroid-based oral contraceptives would have been virtually impossible without plentiful supplies of compounds from the processing of the steroidal components like Dioscin (III) of plants such as yams (Dioscorea sp.) and sisal (Agave sp.).The renaissance of interest in plant products has also been stimulated by the use of plant extracts in chronic conditions for which conventional medicine is perceived to offer very little comfort, e.g., Chinese herbs for severe atopic eczema. Other conditions are hard to define clinically, but "herbal" medicines claimed to alleviate them enjoy huge sales – e.g., ginseng as a tonic and for fatigue. Studies of some of these plants have yielded compounds with unique activity – e.g., ginkgolides, Bilobalide [IV - V], specific platelet activating factor antagonists that were obtained from the Chinese tree Ginkgo biloba[9]. A standardized extract of ginkgo leaves is one of the most frequently prescribed medicines in Germany and is taken to alleviate cerebral ischemia[10].

Amid all these developments it is sobering to realize that less than 10% of the estimated 250,000 flowering plant species in the world have been examined scientifically for their potential in medicine. 60,000 species of higher plants will probably have become extinct by the year 2050, and this accelerating loss of species due to destruction of habitat, as well as loss of knowledge of traditional uses of plants with the attrition of indigenous cultures, strikes a note of urgency into the quest for the new compounds of therapeutic interest.

How should we select plants for serious study? One approach is to consider why it is that plants produce biologically active secondary metabolites, often with highly complex chemical structures. A common view is that such compounds are mainly concerned with survival .This feature can have a positive dimension such as the attraction of pollinators or a more negative aspect such as a feeding deterrent, general toxicity to predators or prevention of growth of competing organisms in the immediate environment. Although chemical protection against the common classes of plant pathogens (viruses and fungi) and insect predators has implications for development of drugs and pesticides, it is difficult to explain the vast array of activity that other secondary metabolites exert on various animal species. Consequently, the search for new drugs has had to rely on other methods, the two most important being random screening of plants for chemicals or activity and ethno pharmacological investigations.

By the middle of the 20th century, certain groups of chemical compounds such as alkaloids were known to be responsible for the biological activity observed in drug-producing plants. This observation led to the screening of other plants for similar compounds that could be detected by simple chemical tests. Few major advances ensued, but this approach furnished many novel structures whose potential as drugs is still not exhausted. The detailed chemical investigation of plants related botanically to those that have already yielded interesting compounds is a logical approach. A good example is the introduction of species of Valeriana other than the traditional V. officinalis, commonly known as valerian, as sources of the tranquilizing valepotriates[11]. Standardized extracts have been widely used in mainland Europe as an alternative to drugs such as benzodiazepines.

The random screening of plants for food and medicine by our prehistoric ancestors is probably the basis of the botanical pharmacopoeia that exists in virtually all cultures. Some early tests for pharmacological activity were done with animal models, but numerous methods have not been introduced for testing of biological activity. The bioassays were made possible by the introduction of molecules biological techniques, with development of receptor-ligand binding assays. Over twenty-five of these receptors are now used routinely by the pharmaceutical industry. This approach is also used by some large government programs e.g., the National Institute of Health screen for anti-HIV agents in this way. Development of new test systems may reveal move towards activities and compounds that could explain the traditional use of a plant previously thought to be inactive. Thus the general immuno-stimulant effect of plants such as Echinacea species, used as a tonic and to prevent infections, was shown only with the advent of tests that could detect an increase in immune response[12].

Ethnopharmacology is the scientific study of plants used by a cultural group for medicinal purposes. As a named discipline, ethnopharmacology is new, but its principles have long been followed in the interchange of information between different cultures e.g., the introduction of drugs such as Senna into western Europe after contact with the Islamic Middle East at the time of the crusades. During the twentieth century the investigation of plants used for generations has resulted in some important therapeutic advances e.g., the introduction of the first tranquilizer, reserpine [VI], from the Indian plant Rauwolfia serpentina, sold in Indian bazaars as a treatment for insanity. Moreover, new activities and chemical substances are still being reported for plants with a long history of medicinal use, the beneficial cardiovascular effects of garlic being one example[13].

Less well-known are some new therapeutically useful compounds that have been isolated from such plants. The Indian plant Coleus forskholii is a source of forskolin [VII], which has unique activator properties on adenylate cyclase and is being developed as a treatment for glaucoma, and artemisinin [VIII], from Artemisia annua, is a promising antimalarial. Artemisinin contains an unusual endoperoxide moiety in its structure and minor modifications have yielded several clinically useful drugs such as artemether . The whole area of antiprotozoal drugs from plants is especially interesting since only in recent years have in vitro tests enabled bioassay-guided fractionation of plants extracts.

CONCLUSION

Research programs in developing countries including India and China are active in the investigation of local medicinal plants. This requires research on ethno pharmacology and ethno botany to a higher extent to maximize the drug screening from these wide biodiversity. A related area of interest is the scientific study of nutraceuticals i.e., plants used for food but also taken regularly as a preventive

measure against disease. The role of phytoconstituents including flavonoids and others with activities such as antioxidant and free radical scavenging is well known, which could be important in the prevention of chronic inflammatory diseases including cancer[14]. There are many possibilities for research, but priority should be given to tropical infectious and chronic diseases for which current mediations have severe drawbacks and to the scientific appraisal of plant-based remedies that might be safer, cheaper, and less toxic for self-medication than existing prescription medicines.

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❑ ❑ ❑

"SOFTWARE ON INFLAMMATORY DISEASES"

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Birla Institute of Technology, Mesra, Ranchi

ABSTRACT

The software has been developed on "Inflammatory Diseases". This software consists of information on twenty such diseases and the drugs used to treat them. The information about the diseases includes their cause, symptoms, diagnosis and treatment. The drugs have been discussed in detail, including their chemistry, indications, contraindications, interactions and adverse reactions. The software is very simple and can be used by Doctors/Pharmacists as a ready reckoner. The software has a provision to add any new disease or drug and also to edit any information about the existing diseases or drugs.

Keywords : Inflammation, Rheumatic diseases, Inflammatory conditions, Anti-inflammatory.

INTRODUCTION

The future practitioners will need improved availability of informations on diseases and drugs. With reference to a particular disease, drugs must be well understood for their appropriate/ inappropriate uses, hazards, interactions, contraindications etc. Fortunately, greatly reduced costs of computing power, and greatly enhanced computer capabilities, offer the promise of extending the services of computerized information and distribution system to every level of medical and pharmacy practice, just as these challenges arise[1].

In the today's world of Information Technology, an attempt has been made to prepare software, which will process the details on diseases and the current drugs, used to treat them. This software will help the practicing doctors and pharmacists as well, to take an instantaneous decision on the disease and its therapy. This will reduce the abuse of various drugs along with making the treatment economical.

It is not easy for a practitioner to remember details of each disease and the drugs used to treat them. It is again impossible to retain all the possible interactions among the drugs and their side effects. So, this software will be quite convenient to a doctor on the diseases and will provide desk-organized information, readily available on their personal computers.

MATERIALS AND METHODS

This software consists of details of few inflammatory diseases and their treatment. Twenty such diseases were selected and their cause, symptoms, diagnosis and treatment are discussed. In the treatment section, the chemistry of drug, indications, contraindications, interactions and adverse reactions are discussed.

The inflammatory diseases discussed in this software are:

Ankylosing Spondylitis, Bronchiectasis, Bursitis, Chronic Hepatitis, Esophagitis, Gastritis, Gouty Arthritis, Inflammatory Bowel Disease, Interestitial Lung Disease, Mixed Connective Tissue Disease, Myopathies, Pancreatitis, Pelvic Inflammatory Disease, Psoriatic Arthritis, Relapsing Polychondritis, Rheumatoid Arthritis, Sjogren's Syndrome, Systemic Lupus Erythematous, Tendinitis, Vasculitis Syndrome, The drug discussed in this software belongs to four categories. These are:

I. Non-Steroidal Anti-inflammatory Drugs

Asprin	Celecoxib	Diclofenac	Ibuprofen
Indomethacin	Ketoprofen	Mefenamic Acid	Meloxicam
Mesalazine	Naproxen	Nimesulide	Oxyphenbutazone

II. Steroidal Anti-inflammatory Drugs

Betamethasone	Dexamethasone	Hydrocortisone	Prednisolone

III. Immunosuppressant Drugs

Azathioprine	Methotrexate

IV. Disease Specific Drugs

Allopurinol	Colchicine	Probenecid	Sulfasalazine
Auranofin	Chloroquine	Interferon Alfa-2A	Serratiopeptidase
Amoxycillin	Cefotaxime	D-penicillamine	Ranitidine
Clindamycin	Erythromycin	Clarithromycin	Sucralfate
Cimetidine	Omeprazole		

The software was developed in Visual Basic 6[2-3]. All the informations about the diseases and drugs were stored in database file of Microsoft Access.[4-7]

The information about the diseases and drugs were obtained from various renowned books on Medical & Pharmaceutical Sciences.[8 - 14]

RESULTS AND DISCUSSIONS

When the software is started the Welcome Screen (Picture 1) is displayed on the desktop. The Menu bar on the software's welcome screen provides access to two commands:

(A) File (B) Search

A) File: The informations about a medicine or a disease can be added to the software by using the File menu. On clicking this menu a pull-down folder appears (Picture 2), which contains the following items:

* Add Medicine * Add Disease * Link * Exit

- **Add Medicine/Add Disease:** On clicking this menu item a new window appears (Picture 3 and Picture 4) on the screen. In this window, information about a new medicine/disease can be added or any new information about the existing diseases or drugs can be edited and updated. The tool bar contains buttons that provide quick access to the commands. The commands are:

Add	:	To add a new medicine/disease
Edit	:	To edit any new information in the existing medicines/diseases
Delete	:	To delete any information in the existing medicine/disease
Refresh	:	To refresh the information after editing or deleting
Close	:	To close the Add Medicine/Add Disease Window

- **Link:** On clicking this menu a new window/dialog box appears (Picture 5). With help of this window, the medicines can be linked to diseases. After linking the medicines to the diseases the data can be saved by pressing the Save button.

- **Exit:** By clicking this menu we can exit the software.

  All the informations, which are filled in the Add Medicine, Add Disease and Link, are saved in the database file Med (Picture 6).

B) **Search:** The information about any of the existing disease can be obtained by clicking this menu. On clicking this menu a new window/dialog box opens (Picture 7), which consists of the list of inflammatory diseases. The disease of choice can be selected from the list and on clicking the GO button another window opens (Picture 8), which consists of the detail information about the disease along with the list of drugs, which can be used to treat the disease.

The detail information on any medicine could be obtained by selecting the medicine and pressing GO button (Picture 9 and Picture 10). If any drug does not exist in the list than a Message box (Picture 11) appears.

- **Home:** On pressing this button we can again reach to the window that contains the list of diseases (Picture 7).
- **Exit:** By pressing this button we can exit the search.

The programming for the software is done in Visual Basic 6.

It is evident from the illustrations that this software is very helpful as well as useful for a practitioner. This software will assist the practitioner in diagnosis of a disease and also to prescribe effective medicine(s) to cure the patient.

This software can further be enriched with more and more informations about the existing diseases or drugs and also newer ones. This will help to make this software, universal software on all diseases.[15]

ACKNOWLEDGEMENT

Authors are thankful to the Head, Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, for providing the facilities to carry out this work.

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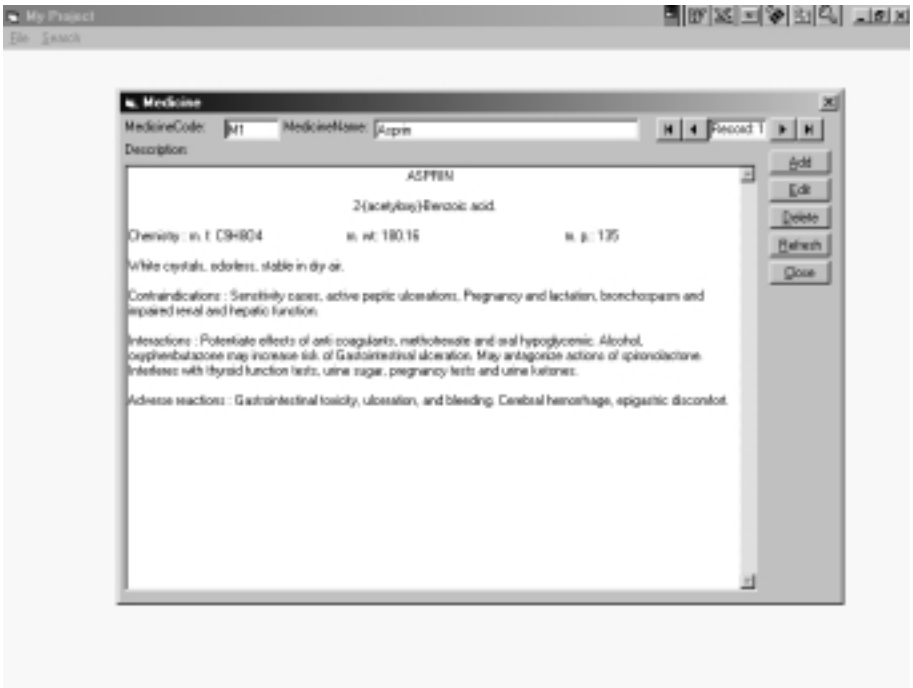
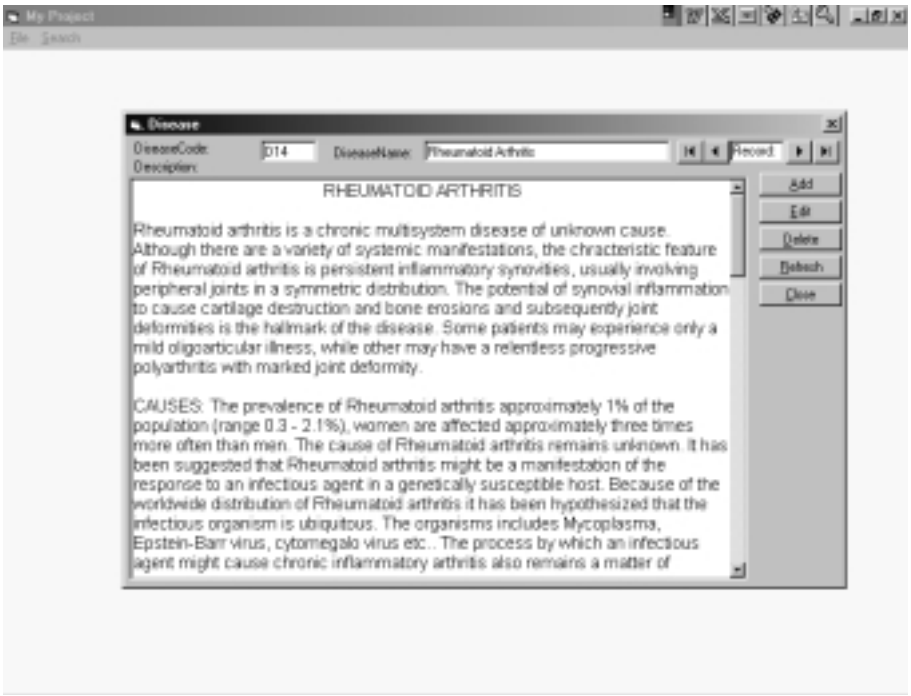
PICTURE - 1

PICTURE - 2

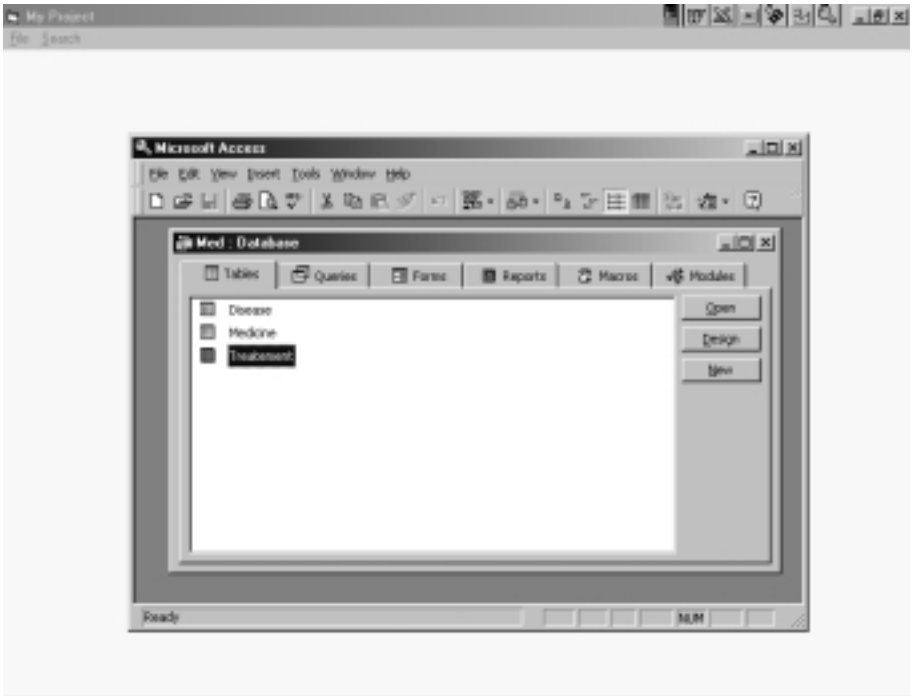


PICTURE - 3



PICTURE - 4

PICTURE - 5



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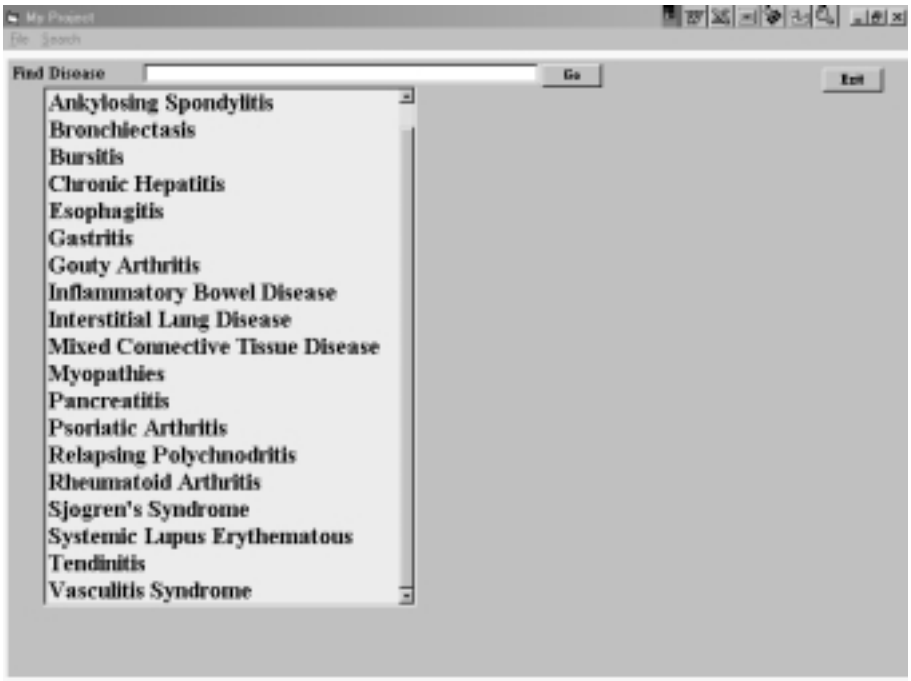
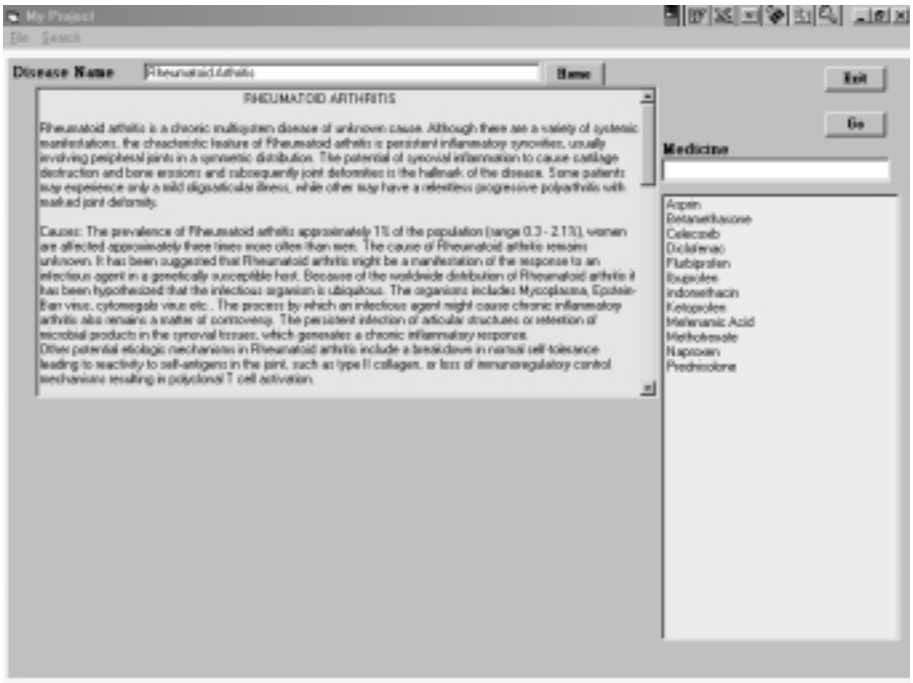
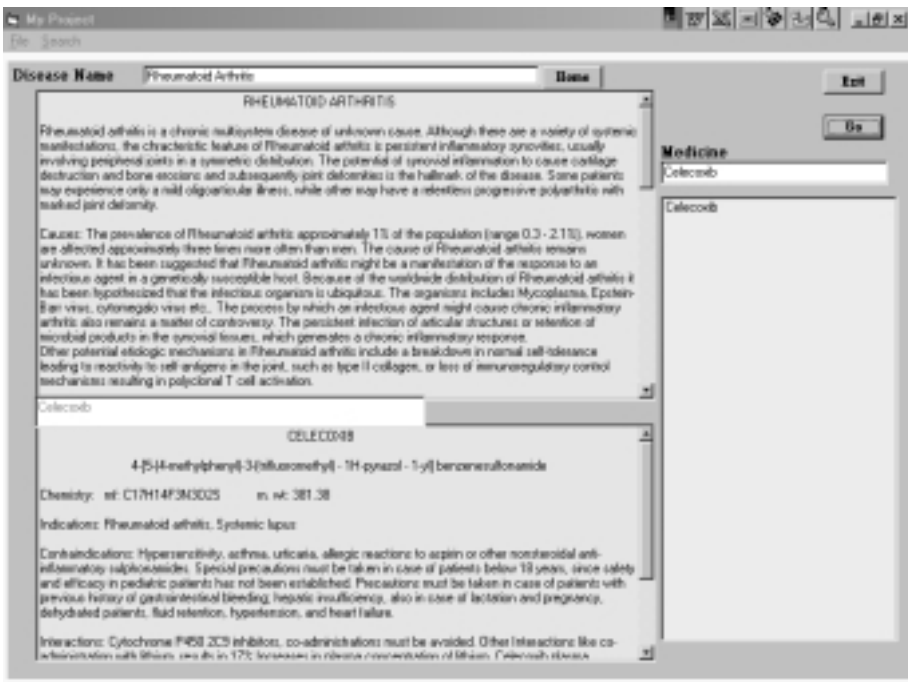


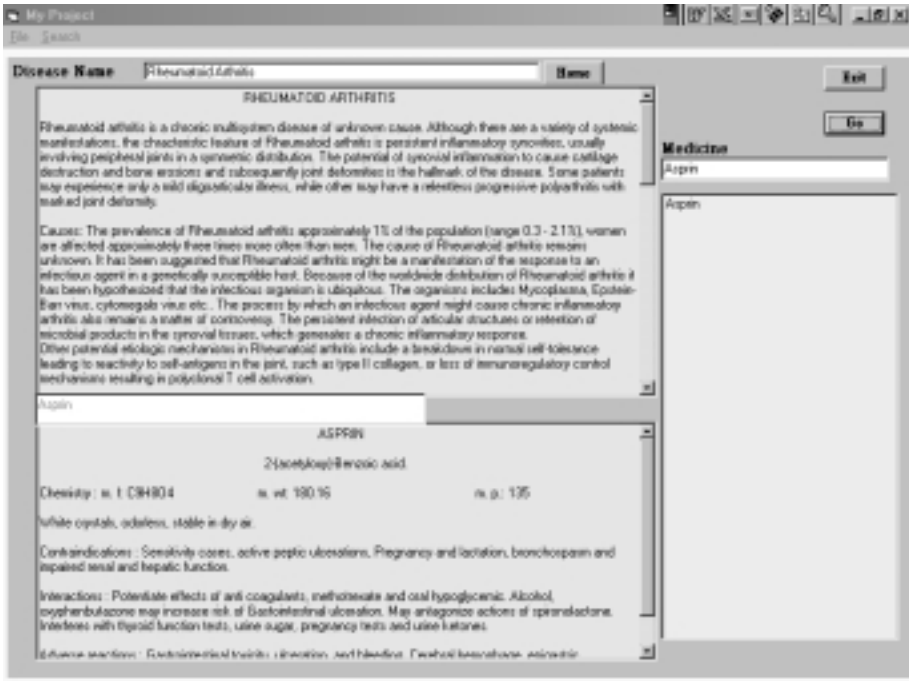
PICTURE - 7



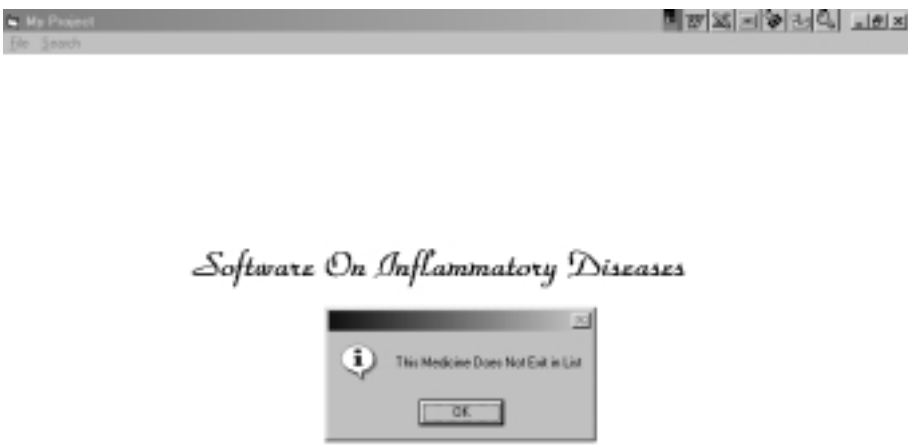
PICTURE - 8



PICTURE -9



PICTURE - 10



PICTURE - 11

KINETICS OF LOVASTATIN PRODUCTION BY MONASCUS PURPUROUS MTCC 369 IN SUBMERGED FERMENTATION

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ABSTRACT

Lovastatin, an HMG-CoA reductase inhibitor can be produced by *Monascus purpureus* MTCC 369 as secondary metabolite and subjected to a complex regulation. Its production was initiated only after assimilation of glucose, moreover fungal biomass does not have any interference on lovastatin production. The onset of lovastatin biosynthesis after dextrose consumption can be attributed to relief from carbon catabolic repression.

Keywords: lovastatin, kinetics, *Monascus purpureus*, submerged fermentation

INTRODUCTION

Lovastatin, a hypocholesterolemic agent, competitively inhibits the rate-limiting enzyme of cholesterol biosynthesis 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the reduction of HMG-CoA to mevalonate during cholesterol biosynthesis[1,2]. Numerous fungi namely, *Monascus ruber*[3], *M. purpureus* [4,5], *M. paxi*[6], *M. anka*[4], *Aspergillus terreus* [6], *Aspergillus flavipes*[7], *A. fischeri, A.flavus, A. umbrosus, A. parasiticus, Accremonium chrysogenum, Penicillium funiculosum, Trichoderma viridae, T. longibrachiatum*[8] have been reported for lovastatin production by submerged and solid state fermentation.

During many fermentation processes the biomass concentration and product formation are influenced by different fermentation parameters such as carbon source level, nitrogen source level, pH of the fermentation medium. The objective of the present study was to investigate the influence of carbon source level and fungal biomass on lovastatin production in a chemically defined optimized medium.

MATERIALS AND METHODS

Microorganism

Cultures of *Monascus purpureus* MTCC 369 obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. It was maintained on Potato-Dextrose Agar (PDA) slants medium at 4°C and sub cultured every 30 days.

Preparation of seed culture

Spore suspension of *M. purpureus* MTCC 369 was prepared from actively growing slants in sterile water and diluted to a concentration 5.7×10^3. 15% spore suspension was inoculated to conical flasks containing the basal medium (100g dextrose, 10g peptone, 2g KNO_3, 2g $NH_4H_2PO_4$, 0.5g $MgSO_4.7H_2O$, 0.1g $CaCl_2$ in 1000 ml distilled water; adjusted to pH 6.0). These cultures were incubated at 30°C for 48 hr in a shaker incubator at 110 rpm[4]

Submerged fermentation

All experiments have been carried out in duplicates in 250 ml Erlenmeyer flasks containing 50 ml chemically defined optimized medium containing 29.59 g/l dextrose, 3.86 g/l NH_4Cl, 1.73 g/l KH_2PO_4,

0.86 g/l $MgSO_4$,$7H_2O$, and 0.19 g/l $MnSO_4$, H_2O, pH of the broths adjusted to 6.0 with 0.1M HCl or NaOH (Sayyad *et al*. 2006). These flasks were autoclaved at 15 psi and 12^0 C for 15 minutes. Then each flask inoculated with 10% seed culture and incubated at 30^0 C for 14 days on a rotary shaker at 110 rpm.

Lovastatin extraction from fermented medium

Fermented medium was sonicated, adjusted to pH 3.0 using 2N H_3P0_4 and extracted with equal quantity of ethyl acetate. The mixtures were centrifuged at 3000g for 8 minutes, 1 ml supernatant was collected and lactonized with 10 ml of 1% trifluoroacetic acid . The resultant was concentrated, diluted to appropriate concentration with acetonitrile and filtered through 0.45ìm filter for HPLC analysis.

Lovastatin estimation

Procedure given by Samiee et al. for HPLC analysis was slightly modified[8]. Lovastatin estimated by HPLC (SHIMADZU, Japan) using 250 mm x 4.6 mm ID Lichrosper® 100 C_{18} column of 5mm particle size and 20ml loop injector. Acetonitrile: Water acidified to the concentration 0.1% with *ortho*-phosphoric acid (65:35 v/v), was used as mobile phase. Flow rate of mobile phase maintained at 1.5 ml/min and detection was carried out by UV-detector at 235nm[9].

Estimation of fungal biomass

Fungal biomass (dry cell weight) was determined by filtering known volume fermented broth through pre-weighed Whatman paper in duplicate. It was then washed with distilled water and dried at 60^0 C under partial vacuum for 10-12 Hrs. Filter papers were then weighed repeatedly after regular intervals to a constant weight[10].

Estimation of residual sugar

The total concentration of residual sugar (glucose) in the fermented broth was measured by the phenol-sulphuric acid method. Broth Sample was appropriately diluted with deionized water. To the 100ml of diluted sample, 1 ml deionized water, 1 ml 5% phenol, and 5 ml concentrated sulphuric acid were rapidly added. This mixture was then incubated at 40^0 C for 20 min. for stable colouring. The total residual sugar concentration was estimated by mesuring the absorbance at 490 nm on specectrophotometer and correlating it with standard. The mesurements were carried out in duplicate for accuracy[11].

RESULTS AND DISCUSSION

In the starting days of fermentation process biomass increases rapidly but after third day of fermentation it grows slowly after that it remains constant. Residual sugar estimated was glucose. Tremendous decrease in sugar concentration was observed from third day. Moreover lovastatin production started from second day (Table 1 and Figure 1).

In filamentous fungi *M. purpureus* MTCC 369 production of secondary metabolite lovastatin is subject to complex regulation. The lovastatin production was initiated only after assimilation of glucose; moreover fungal biomass does not have any interference on lovastatin production, it almost remains steady after dextrose assimilation (Figure 1). Dextrose concentration determined at the starting of fermentation was higher than the added dextrose. It is due to residual glucose coming from inoculated seed culture. The onset of lovastatin biosynthesis after glucose consumption can be attributed to relief from carbon catabolic repression. Similar phenomenon was also observed[2] during lovastatin biosynthesis

Table1

Biomass (dry cell weight) formation, glucose retention and lovastatin production during fermentation process

Time (day)	Dry cell weight (g/l)	Glucose (g/l)	Lovastatin (mg/l)
0	0.61 ± 0.035	53.15 ± 0.354	0.00 ± 0.00
1	2.41 ± 0.028	50.50 ± 0.283	0.00 ± 0.00
2	3.99 ± 0.170	23.65 ± 0.212	10.64 ± 0.09
3	5.49 ± 0.099	3.18 ± 0.078	42.74 ± 0.09
4	5.85 ± 0.085	1.52 ± 0.035	51.14 ± 0.09
5	6.51 ± 0.085	0.76 ± 0.049	58.24 ± 0.08
6	6.64 ± 0.064	0.59 ± 0.035	65.21 ± 0.12
7	7.13 ± 0.049	0.56 ± 0.021	79.70 ± 0.10
8	7.58 ± 0.064	0.53 ± 0.021	116.11 ± 0.18
9	7.73 ± 0.085	0.47 ± 0.021	128.46 ± 0.10
10	8.18 ± 0.078	0.43 ± 0.021	159.58 ± 0.22
11	8.43 ± 0.099	0.33 ± 0.021	209.71 ± 0.35
12	8.84 ± 0.106	0.24 ± 0.021	271.88 ± 0.20
13	8.41 ± 0.092	0.12 ± 0.028	320.30 ± 0.15
14	8.41 ± 0.028	0.08 ± 0.014	326.29 ± 0.11

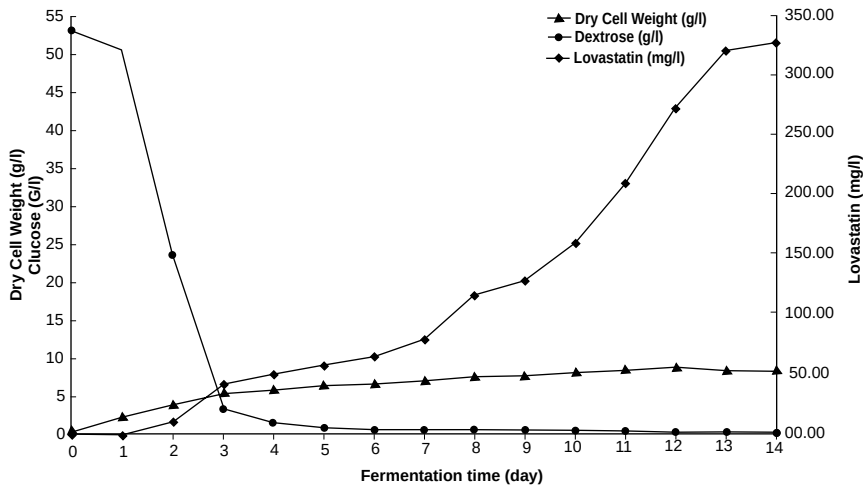

Figure 1 *Change in* glucose *(dextrose) concentration and biomass (dry cell weight) during lovastatin production by Monascus purpureus MTCC 369*

by *Aspergillus terreus* in a chemically defined medium. He found higher specific productivity in defined medium as compare to complex medium with glucose, yeast extract, and peptonized milk. An explanation might be that stringent starvation conditions are required. In complex medium strict starvation conditions may not apply after glucose consumption.

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MOLECULAR DYNAMICS - A VERSATILE TOOL FOR DRUG DESIGN

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ABSTRACT :

Molecular modeling has become a well-established discipline in pharmaceutical research. The essence of an interactive molecular modeling system is the ability for the scientist to explore the conformational variability and properties of the molecules and their interaction with each other. Molecular Dynamics (MD) is a useful tool in this process, thus contributing to rational drug design in a big way. This paper reviews the Molecular Dynamics methods, its advantages and disadvantages and its applications in the field of Drug Discovery and Development on the whole.

INTRODUCTION

Molecular Modeling is a field, which has grown rapidly since 1980s. A number of spectacular advances have been made in molecular biology and in experimental and theoretical structural chemistry as well as in computer technologies. The techniques in molecular modeling provide extensive insight into the precise molecular features like molecular geometries, atomic and molecular aspects and hydrophobic forces that are responsible for regulation of biological processes. One of the important techniques in this field is Molecular Dynamics (MD).

In Molecular Modeling, it is necessary to develop an effective conformational search method in order to get reliable results. Molecular Dynamics is a conformational analysis method, which aims to reproduce the time-dependent motional behavior of a molecule. The molecule is described as a dynamic structure, which changes with respect to time as influenced by its kinetic energy and by the interaction forces of the surrounding atoms. The potential energy function and its associated force field typically include bonded interactions involving bond stretches, and dihedral angle bends, and non bonded interactions including electrostatic and Vander Waals terms. MD provides an opportunity to calculate average properties and conformations of the molecular systems as a function in time. The conformation of the molecule is varied while monitoring the energy of the system by the classical Newtonian equations of motion. Calculation is based on the instantaneous coordinates of all of the particles of the system to evaluate their energies and forces of interaction. Given the coordinates, velocities, and resulting forces, one can compute the coordinates and velocities that the particles would have a short time later. This process is then repeated many times, yielding the motion of all of the particles over the time of the simulation. The small time steps are usually of the order of one femtosecond (10^{-15} s), and a typical simulation might perform ten million of these steps, resulting in a simulation of 10 nanoseconds (10^{-9} s). The maximum size of the small time steps is limited by the fastest motions in the system, which, for biomolecules, corresponds to the vibrations between chemically bonded atoms. An important feature of molecular dynamics is that it provides a means of overcoming conformational barriers, due to kinetic energy present in the system. Kinetic energy is directly related to the temperature of the system, the higher the temperature, the higher the kinetic energy. When searching conformational space it is therefore, a common practice to use high temperature molecular dynamics[1-7].

MOLECULAR DYNAMICS SIMULATIONS INVOLVES 4 STEPS VIZ.,

- The first step is choosing the size, composition and potential energy function of the system used to describe the interatomic interaction. The actual size of the system to be studied will depend on a number of factors and not the least of the computer resources available. Atoms in the finite system are allowed to interact with atoms in the surrounding replicates. The number of solvent molecules in the finite system must be chosen large enough so that the central complex is not too close to any of it's replicates, otherwise concentration effects will occur.
- In the second step, the system must be equilibrated at the temperature of interest. The atomic positions and velocities in the initial model must be adjusted with that found at the desired temperature. It involves the use of both energy minimization and molecular dynamics algorithms. In the equilibration process, usually the solvent surroundings are allowed to relax first while the complex is held fixed, otherwise due to the stresses in the system, the protein conformation may distorted. Then the solvent molecule is subjected to 20ps of MD. At this point, the complex itself is allowed to move. The energy minimization and MD procedures described above are repeated for all the atoms of the system.
- The third step consists of simulations of 20-30 psec (20,000 – 30,000 steps) and in this the atoms are allowed to move in accordance with Newton's equations. The simulations can be carried out in vacuo or in the solution. If the simulations are performed in solution, atoms near the surface will be distorted by vacuum boundary conditions unless edge effects taken into account. The quality of the simulation can be gauged by the similarity of the time-averaged structure of the complex to the original X-ray structure. The RMSD will be less than about 2.5 A° is an ideal one.
- The final step is the analysis of the resulting trajectory by computer graphics or by graphical analysis of variables of interest.

There are many programs to perform MD simulations such as MM2, AMBER, GROMOS, CHARMm, etc .However, one difficulty in molecular dynamics arising from a large configuration space (i.e., a large amount of adjustable parameters) is the fact that the objective function most likely has multiple local minima. It is a challenge for the optimization algorithm to find the global minimum of the target function rather than just a local minimum. Procedures such as the SHAKE algorithm and simulated annealing have been employed to improve this method .Simulated annealing represents one attempt to overcome this problem. This technique involves the assignment of a fixed temperature to the system. Local conformational transitions across barriers in the target function are possible when those barriers are lower than the kinetic energy of the system as specified by the temperature. On the other hand, local conformational changes that decrease the value of the objective function tends to increase the kinetic energy of the system. Therefore a friction or heat dissipation term is included in the equations describing the motions of the atoms (temperature coupling). Ideally, temperature coupling prevents the system from leaving the global minimum configuration but allows it to move away from local minima.

Strong knowledge of the UNIX operating system and some programming skills are required for the usage of a particular MD analysis program.

ADVANTAGES:

- MD can be used to predict about the affinity of one molecule for another (i.e. affinity of a drug for receptor).

- It provides useful qualitative guidelines for modification of the ligand structure, which is likely to enhance affinity. In comparison to vacuum phase molecular mechanics optimizations, MD calculations which include explicit water molecules are able to provide qualitatively correct predictions of relative binding affinities.
- In contrast to the "Static" molecular mechanics approach, Molecular Dynamics can be carried out with multiple low energy conformations that the molecule is capable of adopting.
- MD may also be useful for generating ligand conformations, which are useful to find favorable binding sites for the molecular fragments.
- It provides a means of overcoming conformational barriers, due to kinetic energy present in the system.
- MD is more useful for creating different conformations of molecule which are not conducive to stepwise bond rotation (e.g. cyclic systems), or which would take too long to analyze by that process (large molecules).

DISADVANTAGES:

- The Computational resources required show that Molecular dynamics is not currently regarded as a practical method for examining large number of molecules. Huge time is needed to sample phase space properly and to perform simulation over multiple small windows
- MD also requires a force field description of all molecules to be available, giving rise to potential parameterization problems when applied to a diverse set of molecules.
- MD requires molecules to remain within the same local minima in a limited area of conformational space. Often, an MD trajectory is not able to step over higher energy conformational barriers. The quality of the results from a standard MD simulaton is extremely dependent upon the starting conformation of the system.
- Higher temperature simulations may tend to unfold the receptor structure.
- Large statistical fluctuations are found in calculating internal energies and enthalpies thus making it difficult to extract useful molecular properties.

APPLICATIONS:

- Novel Drug Design by thermodynamic cycle-perturbation method, Free Energy Perturbation method and Brownian reactive dynamics method – This FEP shows how the strength of ligand binding changes as well as the rate of reaction changes if one group on the ligand is replaced by another by considering the relatively small perturbations of structure and energy associated with replacing one chemical group by another. The Brownian reactive dynamics method is used to predict the rate and speed of enzymatic and other reactions and also predict how changes in the reactants will change these rates e.g. Diffusion-controlled reaction of small substrate molecule superoxide catalyzed by superoxide dismutase[3].
- Prediction of novel ligands for molecular targets[3] (e.g.) MDS method was used in the designing of a_2 adrenoceptor antagonist.
- Designing of an enzyme inhibitor and receptor antagonist [3, 8] (e.g) MDS method has been used for the design of Chorismate Synthase-inhibitor and West-Nile virus NS3 serine protease inhibitor. MDS technique has also been used for the design of 4-Phenyl quinoline analogs as potent Angiotensin-II receptor antagonists.

- Elucidation of structure of medium sized proteins. Two important forms of molecular dynamic simulations are thermodynamic and kinetic. Both techniques are important and provide complementary insight into protein structure and dynamics. Thermodynamic questions involve the structure and stability of conformational states of proteins. Experimental techniques, such as X-ray crystallography and nuclear magnetic resonance spectroscopy, provide a partial picture of the folding thermodynamics, which can be complemented by detailed simulations[9]. By understanding what governs the stable states of proteins, proteins of novel structure and function can be designed[10] and function[11]. Kinetic questions are concerned with time-dependent phenomena: specifically, the questions of how proteins fold so quickly, how much time they spend in intermediate states along the folding pathway, and what interactions govern the overall rate of folding are answered.
- Description of many kinds of events involved in drug-receptor interactions, including the salvation and conformational changes required for initial complex formation and any conformational rearrangements that may occur subsequent to binding[3].
- Prediction of changes in the chemical structure of a drug will change the equilibrium constant for binding to a receptor. In this, thermally accessible molecular configurations that are needed in calculations of entropies, enthalpies and other thermodynamic quantities are generated by using MDS[3].
- Redesign of drugs in response to the development of drug resistance due to mutation in the receptor[3].
- Prediction the folding stabilities of globular biopolymers and mutant proteins.[12]
- Prediction and rationalization of the ion-binding selectivity of an organic host molecule in water[13].
- Study of the association between enzymes and inhibitors, of the folding stability of mutant proteins e.g. Binding of substituted benzamidines to trypsin or binding of benzamidines to substituted enzymes[14, 15].
- Study of the catalytic effects of mutations in the enzymes like trypsin and subtilisin[16,17].
- Prediction of the free energy of binding of inert gases like He, Ne, Ar, etc in myoglobin[18].
- Determination of bioactive confirmation of the ligand. (e.g.) Gonadotrophin-releasing hormone (GnRH)[19]

CONCLUSION

Molecular Dynamics approach is a well established method to perform conformational searches of a molecule. Though somewhat complicated, It can also be used for docking and other molecular modeling studies with some additional improvements. Provided the computing time for MD calculations is reduced by help of powerful computers MD can provide valuable insights in Structure Based Drug Design.

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SELF - MEDICATION : PRACTICE CAUTION!

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INTRODUCTION

Self-medication can be defined in simple terms, as "the use of medicines by a person by himself/herself for treating a perceived or real health problem without consulting a physician or pharmacist". The World Self-Medication Industry (WSMI) defines Self-medication as "the treatment of common health problems with medicines especially designed and labeled for use without medical supervision and approved as safe and effective for such use".

The term self medication encompasses a wide array of activities. Over-the-counter drugs are a form of self medication. The buyer diagnoses their own illness and buys a specific drug to treat it. A person may also self-medicate by taking more or less than the recommended dose of a drug. Sometimes the use of illicit drugs, to treat a perceived or real malady, often of a psychological nature, comes under the preview of Self-medication. Often chronic sufferers attempt to correct their illnesses by alternatively trying different sorts of drugs. While this may provide immediate relief of some symptoms, it may evoke and/or exacerbate some other symptoms that are already latently present, and may lead to addiction/dependence, among other side effects of long-term use of the drug. Yet, Self medication is a widely practiced and accepted activity.

WHY DO WE GO FOR SELF MEDICATION?

The most common reasons for choosing to self-medicate could be:

1. The lack of time to go to a physician,
2. Inability to afford physician's fees,
3. Relatives/friends' advice/insistence to buy a particular medicine based on their experience,
4. Increased awareness of availability of medicines and use for appropriate conditions through advertisements, awareness campaigns and information over the Internet,
5. Also, the general tendency is to momentarily suppress the minor symptom/condition rather than wanting to go to the root cause of it because of hidden fears, cost factor, busy schedule etc.

IS SELF MEDICATION AN IRRESPONSIBLE ACTIVITY?

Not always.

Responsible self-medication is when one self-medicates after being well informed about their safe and effective use and their appropriateness for a particular condition.

Responsible self-medication is one of the first lines of self-defence in health care, and being the most accessible form of health care, it fulfills a series of valuable and sometimes crucial functions for individuals and healthcare systems. With responsible self-medication people take greater personal control of their life, with shared decision-making and increased overall self-responsibility. The costs and benefits of self-medication for the individual and society are summarized in the table below:

SELF MEDICATION- COSTS AND BENEFITS TO SOCIETY

	To individual	To society
Costs	▪ Cost of product ▪ Time and cost of transport to obtain product	None
Benefits	▪ Symptomatic relief or treatment. ▪ Less time off work ▪ Usually less expensive than a consultation with a doctor and prescription ▪ The individual becomes more knowledgeable about health and medicines.	▪ No ambulatory care costs. ▪ No drug reimbursement costs ▪ Reduced loss in output from absence from work ▪ Usually no loss in quality of health ▪ Citizens more self-reliant ▪ Health outcomes benefit from reallocation of healthcare resources.

The individual bears the cost of the self-medication product and any time and travel costs associated with obtaining it. Society bears no costs but stands to receive financial and non-financial benefits. Most obviously, the costs associated with a doctor visit and prescribed medicines are avoided. Responsible self-medication offers benefits beyond cost saving. Society benefits from a citizenry that is better informed about healthcare and more self reliant, and from the improvement in overall health outcomes that which would be possible, if public resources currently consumed in treating common ailments were re-directed toward illnesses with a larger impact on overall health.

Taking in account the benefits to individual and the society as a whole, people should keep themselves well informed about the general medicines and treatment aspects and then choose to self medicate. Information can be obtained from various sources. Awareness campaigns by manufacturers relate information about the medicine through pamphlets, product information leaflets and advertisements. However, advertisements focus on attracting attention, offering choices and providing limited general information to mass audiences of consumers and though it is a primary aspect of an information system, it is limited in how much it can reasonably conveyed.

THE 'LAXMANREKHA' OF SELF MEDICATION

Yet, there are considerable hazards in self medication. A large number of people, when they fall sick, do not consult the physician. They either consult a chemist and obtain a medicine from his shelf, or may consult a neighbour who may be having some tablets left over from his previous illness, and readily spares them. If one has a fever, cold and cough, constipation or indigestion, friends or even total strangers volunteer gove advices on medicines like expert physicians. Almost everyone, often has an excellent remedy for the disease and for which he/she often falls for. May be most of the times nothing untoward happens on following such advice, but it can be dangerous.

Another hazard is the availability of many irrational drug combinations in the market, which expose the individual to several drugs needlessly, and may cause adverse effects. Very few combinations have a legitimate place in modern medicine. Yet irrational combinations abound and are being used by some professionals.

Some times the problem that one is facing is due to an underlying illness and self medication will delay the visit to the doctor, making the illness worst.

Paracelsus (1493-1541), the alchemist-physician, in the 16th century observed that all drugs are poisons. The availability of potent and dangerous drugs has increased considerably since the close of the 19th century. At the same time expanding availability of medical care, exposes a large population to drugs, leading to a greater risk exposure to a number of toxic reactions. This situation is further worsened in our country by the slack implementation of Drug Rules. Even certain prescription drugs are available to the lay person without the physician's advice. As people vary greatly in their sensitivity to drugs, an appropriate dose for one person can be an overdose for another. Even Qualified physicians sometimes fail to avoid such reactions. Thus, the lay person is ill-advised in subjecting himself to potentially dangerous self-medication.

Today over 7000 drugs and their combinations are available, many of which have been released for general use, and are sold directly to the public as over-the-counter (OTC). Thus a large number of potent drugs are available to the individual for self-medication. Proprietary drugs which are sold over-the-counter include pain relievers, cough remedies, antiallergics, laxatives, vitamins, tonics, antacids and many others. It is questionable whether the benefits outweigh the potential hazards because they also account for poisonings, allergy, habituation, addiction, and other adverse reactions.

The most misused drugs are the analgesics or pain relievers. In fact, age old, ordinary aspirin is as effective, and even safer than any of the modern analgesics like fenamates, oxicams, or Cox-2 inhibitors like rofecoxib and celecoxib. A probable factor causing lavish prescribing and selling of such drugs is vigorous promotion gimmicks by pharmaceutical firms. Today it may even be difficult to obtain simple aspirin in the market. The physicians have apparently accepted the manufacturer's claims and recommend the "modern analgesics" despite their greater cost. Similarly cough remedies, antiallergics, laxatives, vitamins, tonics, and antacids can lead to serious side effects. Even lavish use of vitamins, especially the fat-soluble (A, D, E, & K) can cause problems. As great English philosopher-physician Sir William Osler (1849-1919) rightly said, "One of the first duties of the physician is to educate the masses when not to take medicines".

MODERATING SELF MEDICATION

In order to limit the potential risks involved in self-medication it is important to visit the physician, who will help in determining the exact cause of problem. But this is not sufficient. The physician can diagnoses the problem and prescribe medicines if required. To avoid or minimize the dangers of self-medication, firstly, common people should be educated about the dangers of indiscriminate use of drugs. Secondly, the physicians should be more judicious in prescribing, and must insist on drugs being supplied by the chemist only on a valid prescription. Thirdly, a proper statutory "Drug Rule" must be implemented, rationally restricting the availability of drugs to the public. These three measures would definitely reduce the incidence of drug-related mishaps and help in maintaining good health of the individual and society.

One should realize the negative aspects of self medication and the importance of consulting a physician before purchasing medicines. Self - medications should only be used to treat minor ailments and it is the sole responsibility of the patient. While self-medication presents a range of benefits to individuals and to society generally, this is applicable as long as it does not shift to the domain of self prescription. Hacking into the area of self-prescription will not only be dangerous but also may fatal

at times. One should always remember that similar symptoms in different people or in the same person at different times may not have same cure. It may require different medicines and only the doctor can decide.

It is thus very important to draw a clear distinction between medicines that are designed for use only under a doctor's supervision – 'prescription' medicines, and those safe and effective for use on the basis of the information available through various sources like product information leaflets, labeling or that available over the internet – 'Over-The-Counter' or 'non-prescription' medicines.

SELF MEDICATION – ROLE OF A PHARMACIST

Before doing self-medication, there are various things one should understand about the medicine.

- The name of the medicine and what it is supposed to do.
- How and when to take the medicine, dose and the duration of the medicine.
- Food, drinks and other medicines that should be avoided while taking the medicine.
- Side effects of the medicine, can it be taken during pregnancy or breast feeding.
- What to do if one miss a dose.
- How to store the medicine.

This is where the role of the health care professionals, especially pharmacists comes into play. They can provide valuable information about medicines. Using medicines in the right way is very important to the health of the individual. Only with proper use of medicines, one can get its full benefits.

Considering the lack of time on the part of a doctor and the fees associated for advice, a casual talk with pharmacist can give not only relevant information but also tips on where to look for reliable information. If any one are not having information to choose an appropriate OTC medicine for their condition, they may ask pharmacist for product information leaflet or just a reliable website to look for information. Also, if needed one can ask pharmacist to clarify any doubts that he might have.

As an easily accessible health professional and the person any one can communicate with ease, the Pharmacist can assist in choosing a self-medication for ailment. Pharmacist takes all the care to make sure get the best health care. Well, and what's more, all this without any added professional fee!

It is recommended that only one pharmacy is used where the service obtains medicines on behalf of service users. This will enable the pharmacist to maintain a patient medication record, which will ensure a continuity of care when supplying medication.

When taking someone else's prescription to the pharmacy to be made up, it is important that Pharmacist should check the following:

- the name and address
- the details of the medicine and dosage
- the service record of what has been prescribed
- the prescription signed by the prescribing doctor

The Ministry of Health in UAE has issued a circular, warning the pharmacists not to sell any medicines without proper prescription. This move will certainly help to reduce the self medication problems.

As it is known that prescription medicines are those medicines which are available to the patient only on prescription from a physician.

The pharmacist should guide about medicines and do patient counselling to avoid medication error. Also they should maintain patient's file and updates it everytimes.

SELF MEDICATION IN INFORMATION AGE

Interacting with health care professionals can play a vital role in finding reliable sources of information and in making decisions related to self-medication. Information on reliable websites over the internet can also provide considerable guidelines on the safe and effective use of medicines, what the medicine is supposed to do, who should or should not take it, and how to use it.

CONCLUSION

Today people have to take primary responsibility of their own health. Patients are ready and willing to play a greater part in the treatment of their own diseases and illness. Increased knowledge among people has given them a chance to be responsible for their own medication. Medicines today can restore health and improve the quality of life; on the other hand if it is not used correctly, it can cause serious harm to human being.

Before doing self-medication, there are various things one should understand about the medicine. The name of the medicine and what it is supposed to do; how and when to take the medicine; it's appropriate dose and the duration of effect of the medicine.

Many people visit hospital and fail to get better because they do not take or use their medicines properly.

This is where the role of the health care professional proves important. Pharmacist can give better information about medicines. Using medicines in the right way is very important for health. Only with proper use of medicines, one can get the medicine's full benefits.

Also one should realize the negative aspects of self medication and the importance of consulting a pharmacist before purchasing medicines. Self - medications should only be used to treat minor ailments and it is the sole responsibility of the patient. Self medication can mask the symptoms. Remember, medicines can help only if for the right cause. So one should take a physician or pharmacists advice and should avoid self-medication.

FURTHER READING

www.aesgp.be
www.ameinfo.com
www.schoolhealthservicesny.com
www.boloji.com
www.paho.org

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THE CURRENT STATUS OF SOLID LIPID NANOPARTICLES

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ABSTRACT:

Solid lipid nanoparticles (SLNs) hold great promise for reaching the goal of controlled and site specific drug delivery. SLNs have attracted increasing attention during recent years, particularly as targeted carriers of biomolecules. This article presents an overview of SLNs briefly reviewing the ingredients, production technologies, physicochemical characterization and therapeutic applications of the delivery system. Lipids of different types and appropriate analytical methods are needed for the production and characterization of SLNs. Possible alternative structures and dynamic phenomena at the molecular level need to be considered in SLN development. Aspects of SLN route of administration and their biodistribution are also discussed. Finally, the varied applications of SLNs as reported in public literature have been emphasized. If appropriately investigated, solid lipid nanoparticles have the potential to open new vistas in therapy of complex diseases.

Keywords : Solid lipid nanoparticle (SLN), Colloidal drug carriers, Homogenization, TEM, PCS, biodistribution, drug targeting

INTRODUCTION :

Pharmaceutical dosage forms have evolved over the years from simple pills to complicated dispersed systems. In this gamut of delivery systems, the colloidal drug delivery systems have a very special place. Colloidal particles ranging in size between 10 – 1000 nm are known as **nanoparticles**. They are manufactured from synthetic/natural polymers and ideally suited to optimize drug delivery and reduce toxicity. Over the years, they have emerged as a viable substitute to liposomes as drug carriers. The developments in nanoparticles have been made mainly in the field of polymeric nanoparticles wherein such polymers as poly(lactide-co-glycolic acid) (PLGA), poly-e-caprolactone (PCL), alkyl cyanoacrylates, etc. are used. However, the scarcity of safe polymers with regulatory approval and their high cost have limited the wide spread application of nanoparticles to clinical medicine.[1]

To overcome these limitations of polymeric nanoparticles, lipids have been put forward as alternative carrier of, particularly, lipophilic pharmaceuticals. These lipid nanoparticles are termed as solid nanoparticles (SLN), which are attracting attention of formulators world-wide. The successful implementation of nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometer size.[2]

Solid lipid Nanoparticles are colloidal carriers developed in the last decade as an alternative system to the existing traditional carriers (emulsions, liposomes and polymeric nanoparticles). They are a new generation of submicron-sized lipid emulsions where the liquid lipid (oil) has been substituted by a solid lipid. SLN offer unique properties such as small size, large surface area, and the interaction of phases at the interfaces, and are attractive for their potential to improve performance of pharmaceuticals, neutraceuticals and other materials.

SLNs are also attracting major attention as novel colloidal drug carrier for intravenous applications. The SLNs are sub-micron colloidal carrier which is composed of physiological lipid, dispersed in water

or in an aqueous surfactant solution. It has advantages of good tolerability, scalability to large-scale preparation, excellent biocompatibility and protection of incorporated drugs against chemical/ enzymatic degradation. For lipophilic drugs, they also offer higher loading capacity compared to the polymeric nanoparticles. Being composed of physiological lipids, the end products of metabolism of these particles are safe and biocompatible. They are, therefore, suitable for delivery of drugs belonging to several therapeutic segments. SLNs are poised to open new avenues in research and therapy.[2]

ADVANTAGES OF SOLID LIPID NANOPARTICLES :

SLNs combine the advantages of several colloidal carriers and avoid many drawbacks of such dispersed systems. [3] Some of their prominent advantages are listed below -

1. SLNs have the ability to control and/or target drug release at the desired site of the body.
2. They can improve stability of pharmaceuticals by localizing the drug in their matrix.
3. SLNs show higher drug entrapment efficiency (compared to other carriers), particularly for lipophilic drugs.
4. They may be engineered to carry both lipophilic and hydrophilic drugs.
5. SLNs have very good biocompatibility since most lipids are biodegradable and upon metabolism they yield physiologically acceptable metabolites.
6. They are primarily manufactured through water based technology, thus avoiding organic solvents. Hence, they comply with environmental regulations.
7. They are Easy to scale-up since the equipment used in their manufacture (most commonly the high pressure homogenizer) come in different sizes suitable for lab-scale, pilot-scale and production-scale manufacture.
8. SLNs are more affordable than the polymeric nanoparticles since the physiological lipids are comparatively cheaper than the biodegradable polymers.
9. They are easily sterilizable.
10. They are amenable to lyophilization, and hence their shelf-life may be extended over a considerable period of time.
11. They have been found to be easier to validate and gain regulatory approval relatively quickly.
12. They offer all advantages of other particulate systems such as environmental protection, slow drug release and targetability of the particles through surface modifications.

COMPOSITION & STRUCTURE :

SLNs are made up of solid lipids, emulsifier and water/solvent. The lipids used may be triglycerides (e.g. tri-stearin), partial glycerides (e.g. Imwitor), fatty acids (e.g. stearic acid, palmitic acid), and steroids (e.g. cholesterol) and waxes (e.g. cetyl palmitate) and their combinations. Various emulsifiers (Pluronic F 68, F 127, Tweens) and their combinations have been used to stabilize the lipid dispersion. The combination of emulsifiers might prevent particle agglomeration more efficiently.

The SLNs may come in various forms – the matrix type particles, lipid coated systems, the drug-lipid conjugates (LDC), the nanostructured lipid carriers (NLC). Depending on their internal morphology and energy state of the lipid polymorph the characteristics of the particles varies. The lipid polarity

determines the surface charge and zeta potential and hence the degree of particle agglomeration and stability of the systems. The surface charge and its magnitude also influences the biological distribution of SLNs to various parts of the body. The thermal methods of analysis such as TGA and DSC as well as XRD are, therefore, essential tools to characterize the structure of the SLNs. The correct blend of different lipids and other additives needs to be optimized for a particular drug for a successful SLN formulation. Besides, these instrumental methods of analysis help in explaining the drug release mechanism and prediction of the in vivo performance of the delivery systems.

METHODS OF SLN PREPARATION :

To date, several techniques of SLN manufacture have been reported in research literature and patents. The different methods of SLN manufacture may be broadly grouped as under -

By far the most popular and industrially feasible method is the high shear homogenization technique using high pressure homogenizers (like Micron LAB 40/60) and in some cases the spray drying. Because very high energy density is reached in the high pressure homogenizers, lipid can reach very small size

a. High shear homogenization[4,5]
    1. Hot homogenization
    2. Cold homogenization
b. Ultrasonication[6]
    1. Probe ultrasonication
    2. Bath ultrasonication
c. Emulsification solvent evaporation[7,8]
d. Microemulsion based SLN preparations[9,10]
e. High speed homogenization[11]
f. Spray drying.
g. Double Emulsification technique

of the order of nanometers. The high pressure homogenizers are available at various production capacities like 20L, 40L and 60L/hr which are suitable for laboratory, pilot-plant and production scale manufacture of SLNs. Further, these instruments are adaptable to GMP validation protocols, and hence can be easily adopted to existing production lines. These homogenizers are purported to yield SLNs of very narrow size distribution through an entirely aqueous solvent based technology. Industrial spray dryers can be easily applied for production of fine spherical SLNs. Thus, both these methods are adaptable to large-scale production. On a lab scale, the microemulsification technique may be more preferred due to its ease and simplicity. Microemulsions are optically clear systems and their usual globule size range is below 100 nm. Hence, the SLNs prepared from microemulsions of molten solid lipids thermodynamically achieve very small size in the range of few hundreds of nanometers. Other methods such as the ultrasonication, high speed homogenization, emulsification / double emulsification and solvent evaporation techniques are mainly of academic interest.

SLNs need to be purified of any unentrapped drugs after preparation. The purification may be carried out by several reported techniques such as ultrafiltration, diafiltration, dialysis, gel filtration and ultracentrifugation.

CHARACTERIZATION OF SLN QUALITY AND STRUCTURE:

Adequate characterization of the SLNs is a necessity for the control of the quality of the product.[12] However, characterization of SLN is a serious challenge due to the colloidal size of the particles and the complexity and dynamic nature of the system. Pharmacopoeias across the globe have not included any specific evaluation criteria for the nanoparticles yet. Hence, the evaluation and quality control of

nanoparticles and SLNs remain mainly an in-house exercise, with varied tests and standards followed by different research groups and R&D houses. Often the drug in question and the intended application / route of administration determines the type of characterization required.

Most important physicochemical parameters which need to be studied for the SLNs are -

1. Particle size & specific surface
2. Size distribution kinetics
3. Surface charge and zeta potential.
4. Degree of crystallinity and lipid polymorphism.
5. Coexistence of additional colloidal structures (micelles, liposome, super cooled, melts, drug nanoparticles)
6. Yield, Drug content and drug entrapment efficiency.
7. In vitro drug release.
8. Surface morphology.

There may be several other parameters studied on a case-to-case basis.

Particle size of colloidal systems is the most important parameter in their use as drug delivery systems, particularly when administered parenteral. Ideally, the SLN particle size must not vary appreciably during the storage period. However, they do undergo aggregation, and the biggest challenge to the formulation scientist is to develop such a system, which remains within a narrow size range throughout the shelf-life. The size of nanoparticles have been found to have significant effect on its body distribution, localization and drug release kinetics. The particle size/size-distribution may be studied using photon correlation spectroscopy (PCS), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The surface and internal morphology may be studied using atomic force microscopy (AFM), SEM, Scanning Tunneling microscopy (STM), or Freeze fracture electron microscopy (FFEM).

Zeta potential is an important product characteristic of SLNs since its high value is expected to result in deaggregation of particles in the absence of other interfering factors such as steric stabilizers or hydrophilic surface appendages. It is measured by the electrophoretic mobility of the particles using a zetameter (such as Malvern Zetasizer nano ZS). The surface charge depends on the polarity of the lipid and hydrophilic head group of the surfactant used as dispersion stabilizer. Using lipids of desired polarity, excellent SLNs may be produced, whose size distribution remains almost static over the entire shelf-life.

Polymorphic transitions of the lipid during production and storage of SLNs is important parameters influencing drug entrapment and release characteristics. Differential scanning calorimetry (DSC), Raman Spectroscopy and X-ray diffractometry (XRD) are the major techniques used to characterize crystallinity of SLNs. Synchrotron irradiation may be a better but less accessible technique. Structural details of SLNs may be obtained through IR/Raman Spectroscopy, as well as mass spectroscopy (MS) or NMR. Nuclear magnetic resonance (NMR) and electron spin resonance (ESR) spectroscopy may be employed to detect presence of other colloidal vesicles (like micelles, liposomes, drug nanocrystals) and their dyanamic changes.

The in vitro drug release is usually studied using dialysis or diffusion principles in various kinds of diffusion cells or using dialysis bags. The in vitro drug release profile is often one of the most influential criteria in the success of a SLN formulation.

Apart from the in vitro studies, the SLNs are also studied in animals / humans as in cases of other novel delivery systems. Animal studies are particularly useful in gaining insight into the body distribution and localization profile of the SLNs and the pharmacokinetics of the drug in SLN delivery systems. Such results, however, vary according to the route of administration and type of lipids used in the system.

ROUTES OF ADMINISTRATION AND BIODISTRIBUTION:

The in-vivo fate of the solid lipid nanoparticles depends mainly on the administration route and distribution process - adsorption of biomolecules on the SLN surface and desorption of SLN components into the biological milieu.

SLNs are composed of physiological lipids, their derivatives or waxes. Therefore, transportation and metabolism pathways present in the body may contribute to a large extent to the in-vivo fate of the SLN. The most important enzymes of SLN degradation are lipases (present in various organs and tissues), which split the ester linkage and form partial glycerides or glycerol and free fatty acids. Most lipases require activation by an oil/water interface, which opens the catalytic center. In vitro experiments indicate that SLNs show different degradation rates by the lipolytic enzyme pancreatic lipase as a function of their composition, i.e., type of lipid matrix, stabilizing surfactant, etc. Several routes of administration of the SLNs have been reported and an enumeration of all such reports is beyond the scope of this review. Some of the most important ones are briefly discussed below.

1. PER ORAL ADMINISTRATION[13]

Per oral administration forms of SLN may include aqueous dispersions (nanosuspensions) or SLN loaded traditional dosage forms, e.g. tablets, pellets or capsules.

The microclimate of the stomach favors particle aggregation due to the acidity and high ionic strength. Very little data is available publicly regarding the effect of food on SLN performance. They are expected to be effective irrespective of the fed or fasted conditions. The influence of gastric and pancreatic lipases on SLN degradation in-vivo needs to be studies in greater details.

Unfortunately, very few in-vivo studies have been conducted on SLN yet. Recently, camptothecin (CA) containing SLN were produced from stearic acid (2%), lecithin (1.5%) and poloxamer 188 (0.5%) showed body distribution mainly to the brain after oral administration indicating their utility in targeted sustained release systems.[14]

2. PARENTERAL ADMINISTRATION[14]

SLN have been administered intravenously to animals. Pharmacokinetic studies of doxorubicin incorporated into SLN showed higher blood levels in comparison to a commercial drug solution after i.v. administration in rats. As regards distribution, SLN were found to have higher drug concentrations in lung, spleen and brain, while the solution led to a distribution more into liver and kidneys.

Yang[13] reported the pharmacokinetics and body distribution of camptothecin after i.v. injection in mice. In comparison to a drug solution SLN was found to give much higher AUC / dose and mean residence times (MRT) especially in brain, heart and RES. The highest AUC ratio of SLN to drug solution among the tested organs was found in the brain.

3. TRANSDERMAL APPLICATION[15]

The smallest particle sizes are observed for SLN dispersions with low lipid content (up to 5%). Both the low concentration of the dispersed lipid and the low viscosity are disadvantageous for dermal

administration. In most cases, the incorporation of the SLN dispersion in an ointment or gel is necessary in order to achieve a formulation that can be administered to the skin. The incorporation step implies a further reduction of the lipid content of the SLN dispersion resulting in semisolid, gel-like systems, which might be acceptable for direct application on the skin. Use of SLNs has been reported to have targeting potential to the different layers of the skin, without exposing the systemic circulation to the particles.

APPLICATIONS :

Solid lipid Nanoparticles possesses better stability as compared to liposomes. This property may be very important for many modes of targeting. SLNs form the basis of colloidal drug delivery systems, which are biodegradable and capable of being stored for at least one year. They can deliver drugs to the liver in-vivo and in-vitro to cells, which are actively phagocytic. Furthermore, the SLN production can be easily upgraded to large-scale production. There are several potential applications of SLNs some of which are given below:

1. ***SLNs as gene vector carrier:*** SLN can be used in the gene vector formulation. DNA and siRNA delivery has been achieved using SLNs.[16]
2. ***SLNs used topically:*** Tropolide (TP), a poor water water-soluble drug, has an antiinflamatory, antifertility, antineoplastic and immunosuppressive activity. When TP was incorporated with soya lecithin in SLN hydrogel formulation, the safety was improved and toxicity minimized. Several other drugs including antifungals and oil soluble vitamins have been formulated in SLNs.[17]
3. ***Application of SLNs in cosmetics:*** For cosmetology the SLNs are the novel delivery system. The SLN was applied for the preparation of sunscreens and as an active carrier agent for molecular sunscreens. The in vivo study shows 31% of skin hydration will be increased after 4 weeks by addition of 4% SLN to a conventional cream. They also have been put to use in targeted delivery of drugs to upper layers of skin for vitamin A.[18]
4. ***SLNs for potential agriculture application:*** Essential oil extracted from Artemisia arboreseens L when incorporated in SLN, were able to reduce the rapid evaporation compared with emulsions. So the systems used as ecological pesticides, a suitable carrier in agriculture.[19]
5. ***SLNs can be used as a carrier for anticancer drug to solid tumours:*** Tamoxifen, an anticancer drug incorporated in SLN to prolong release of drug after i.v administration in breast cancer and enhanced the permeability and retention (EPR) effect. Drugs such as camptothecin and methotrexate has been reported as payloads of SLNs.[20]
6. ***SLNs in breast cancer and lymph node metastases:*** Mitoxantrone loaded SLN local injections were formulated to reduce the toxicity and improve the safety and bioavailability of drug.[21]
7. ***Oral SLNs in antitubercular chemotherapy:*** Anti tubercular drug like rifampicin, isonizide, pyrazinamide loaded SLN systems were able to decrease the dosing frequency and improve patient compliance. The nebulization in animal by incorporating the above drug in SLN also reported for improving the bioavailability of the drug.[22]
8. ***SLNs in drug targeting:*** They have appeared as promising carriers in tissue/cell-specific homing devices for drugs with/without pegylation to make them stealth and thus bypass the opsonization mechanism.

Apart from these specialized applications, a PUBMED search reveals that a multitude of drugs of various therapeutic groups and diagnostic agents have been reported to be loaded on SLNs for various reasons. From 1975 onwards the research reports deals with the following drugs in SLN dosage form - (188)Re, (99m)Tc, 10-hydroxycamptothecin, 17beta-estradiol hemihydrate, 5-FU, acyclovir, alpha-lipoic acid, amphotericin B, annexin V, ascorbyl palmitate, beta-elemene, BSA, butyric acid derivative, Camptothesin, cisplatin, clobetasol, cloricromene, clotrimazole, clozapine, cyclosporine, dextran, Diazepam, doxorubicin, etoposide, flurbiprofen, glucocorticoids, griseofulvin, histidine, hydrocortisone, ibuprofen, idarubicin, indinavir, indomethacin, isoniazid, isotretinoin, ketoconazole, ketorolac, Lidocaine, methotrexate, mitoxantrone, N,N- diethyl-m-toluamide, n-dodecyl-ferulate, NFkappaB, nile red, paclitaxel, pilocarpine, podophyllotoxin, prednisolone, pyrazinamid, Retinol, rhodamine B, rifampicin, salbutamol, silibinin, silymarin, tamoxifen, Tashinone IIA, tetrandrine, tobramycin, tocopherol, Ubidecarnone, vinpocetine, and Vitamin K. Although this may not be the complete list, yet it provides an idea of the wide spectrum of applications and potential future implementation areas for the SLNs.

CONCLUSION AND FUTURE PROSPECTS:

In the early days of the 20th century, Paul Ehrlich envisioned his magic bullet concept- the idea that drugs reach the right site in the body, at the right time and at right concentration. It should not exert side effects, neither on its way to the therapeutic target, nor at the target site, nor during the clearance process. These systems have in common that they are indicated for the treatment of life-threatening disease like cancer, and severe infectious diseases and therefore, contribute considerably to our therapeutic armamentarium. The SLNs have the potential to achieve these broad objectives, provided some of their minor in vivo toxicity concerns are addressed[23].

With growing interest in gene delivery and targeting, pharmaceuticals getting more complicated every day; the SLNs have great promise to emerge as one of the foremost drug delivery systems of the new millennium.

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FORMULATION AND IN-VITRO EVALUATION OF ETHYL CELLULOSE MICROSPHERES CONTAINING ANTI HIV DRUG

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ABSTRACT

Microspheres containing the anti HIV drug (Indinavir Sulfate) were prepared by the emulsion solvent evaporation method using ethyl cellulose as wall materials. In order to increase the encapsulation efficiency, a mixed solvent system comprising 1:1 proportions of acetonitrile and dichloromethane was used as a dispersed phase. The influence of formulation factors (stirring speed, surfactant concentration and polymer to drug ratio) on particle size, encapsulation efficiency and in vitro drug release characteristics of the microspheres were investigated. The morphology of the microspheres was evaluated using a scanning electron microscope, which showed a spherical shape with rough surface. The stable nature of drug within the formulations was confirmed by infrared spectroscopy The mean sphere diameter was between 324 to 434 μm and the microencapsulation efficiencies ranged from 69 to 90%. The encapsulation efficiencies and release of Indinavir Sulfate was influenced by the drug to polymer ratio and particle size. The release of drug from formulations was found to be diffusion controlled.

INTRODUCTION

Microencapsulation is a well known method that is used to modify and delay drug release from pharmaceutical dosage forms. The solvent evaporation method with water as an external phase is popularly used for microsphere preparation, for scale-up problem and cost considerations, but this method is not suitable for hydrophilic drugs which is likely to preferentially partition out into the aqueous medium, leading to low entrapment efficiency, when encapsulated using aqueous phase as the processing medium.

In the above study a mixed solvent system (MSS) has been used as a dispersed medium with a suitable non-aqueous processing medium to enable formation of oil-in-oil emulsion. Components of the MSS can be selected from any of the commonly available organic solvents such as dichloromethane, ethyl acetate, acetone, acetonitrile, ethanol, etc.[1-3]. Having chosen oil as the processing medium, it is imperative that the solvent for polymer be immiscible with oil. Acetonitrile is a unique organic solvent, which is polar, water miscible and oil immiscible [4]. All other polar organic solvents like methanol, ethyl alcohol, ethyl acetate, acetone, dimethylsulphoxide and tetrahydrofuran are oil miscible and do not form emulsions of the polymer solution in oil[5]. Polyhydric alcohols (low molecular weight polyethylene glycol, glycerol etc.) or other non-aqueous liquids can also be used instead of oil as the processing medium. With oil as a processing medium, use of acetonitrile alone as a dispersed medium did not ensure formation of a stable emulsion, and a non-polar solvent such as dichloromethane was included to decrease polarity of the acetonitrile solution. In this study, the mixed solvent system comprising 1:1 proportions of acetonitrile and dichloromethane was used.

Literature study shows that there have been few studies about a mixed solvent system as a dispersed medium using ethyl cellulose (EC) as a wall material. EC is a water insoluble polymer and widely used

in pharmaceutics as a wall material for sustained release microcapsules[6]. This is due to its high safety, good stability, easy fabrication and cheapness.

The model drug Indinavir sulphate, (IS) an inhibitor of the human immunodeficiency virus (HIV)[7] protease is the FDA approved drug for treatment of HIV. It is typically administered orally as capsule and oral solution. The virustatic drug has a very short half life (1.80 h).

Therefore, the purpose of the present study is to use the emulsion solvent evaporation method in order to prepare microspheres using EC and to investigate the characteristics of the microspheres.

EXPERIMENTAL

MATERIALS :

Indinavir sulfate was obtained as a gift sample from Macleods Pharmaceuticals Ltd. Mumbai, India. Ethyl cellulose (EC) was purchased from Central Drug House (Mumbai, India). All other reagents and solvents used were of pharmaceutical or analytical grade.

METHODS :

Microspheres were prepared by using oil-in-oil (o/o) emulsion solvent evaporation method, using different ratios of drug to polymer ratio (1:1, 1:2, 1:3 and 1:4) EC and IS was added to the mixed solvent system consisting of acetonitrile and dichloromethane in a 1:1 ratio and mixed thoroughly by a magnetic stirrer. Then the polymeric phase was slowly added to 50 ml of light liquid paraffin containing 10 ml n-hexane and 0.25 % V/V span 80 (HLB value of 4.3) as a surfactant while stirring at 500 rpm and the stirring were continued for a further 2 to 3 h. Then the hardened microspheres were collected by filtration and washed with three portions of 50 ml of n-hexane and air dried for 12 h. Batches were prepared in triplicate to obtain reproducible results.

SIZE DISTRIBUTION OF MICROSPHERES :

Microspheres were separated into different size fractions by sieving for 10 minutes using mechanical sieve shaker (Cuprit Electrical Co. India) containing standard sieves having apertures of 710, 500, 355 and 250 μm[6] .The particle size distribution of the microspheres for all the formulations was determined and mean particle size of microspheres was calculated.

DRUG ENTRAPMENT EFFICIENCY :

About 50 mg of accurately weighted drug loaded microspheres were added to 50 ml of triple distilled water (TDW). The resulting mixture was kept shaking on mechanical shaker for 24 h. Then, after the solution was filtered (0.45 μm pore size) and analyzed spectophotometrically at 259 nm using Systronic 2101 UV - Visible spectphotometer after appropriate dilution.

SCANNING ELECTRON MICROSCOPY (SEM):

The external morphology of the microspheres was analyzed by JEOL JSM - 5200, scanning electron microscope at 20 kV. The microspheres were fixed on a metallic support with a thin adhesive tape and microspheres were coated with gold under vacuum (fine coat, ion sputter JFC - 1110) to render them electron conductive.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR):

Drug polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug, drug loaded microspheres and blank microspheres using FTIR JASIO (Model No. 410). Samples

were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400 to 4000 cm-1 and the resolution was 2 cm-1.

IN VITRO RELEASE STUDIES:

Drug release from microspheres was determined using a basket in an eight station dissolution apparatus (LABINDIA, Disso-2000, Mumbai, India). Microspheres (100 mg) from size fraction 355 mm size were put in each basket. The dissolution medium was 500 ml TDW. The dissolution medium was stirred at 100 rpm and maintained at 37 ± 0.5^0C Samples of 10 ml were taken at aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. After suitable dilution, the samples were analyzed spectophotometrically at 259 nm.

RELEASE KINETICS :

The mechanism of drug release was found out by Korsmeyer Peppas model. The first 60% of drug release was fitted to the following formula[8].

$$Mt / M\mu = Ktn$$

Where Mt / $M\mu$ is the fraction of drug released at time t and k is the release rate constant and 'n' is the release exponent. The 'n' value is used to characterize different release mechanisms and is calculated from the slope of the plot of log of fraction of drug released vs. log of time.

RESULTS AND DISCUSSION

The morphology of microspheres was determined by SEM (Fig.1), which shows that the microspheres were spherical in nature with rough surface. The mean particle size ranged from 324.59 to 434.95 μm (Table 1). The mean size was influenced by various manufacturing parameters like drug to polymer ratio, surfactant concentration and stirring speed. Polymer concentration had a positive effect on mean particle size. The increase in the viscosity with increase in polymer concentration leads to an increase of the emulsion droplet size and finally a higher microsphere size. The minimum concentration of span 80 required to form the stable emulsion was found to be 0.25 % and, as the concentration increased from 0.25 % to 0.5 % the mean particle size was reduced, this was in accordance with the theory of effect of surfactant concentration on particle size[9-10]. When the stirring speed was decreased from 500 to 250 rpm, the mean particle size of the microspheres was increased and when the speed was increased from 500 to 1000 rpm, the mean particle size of the microspheres was decreased.

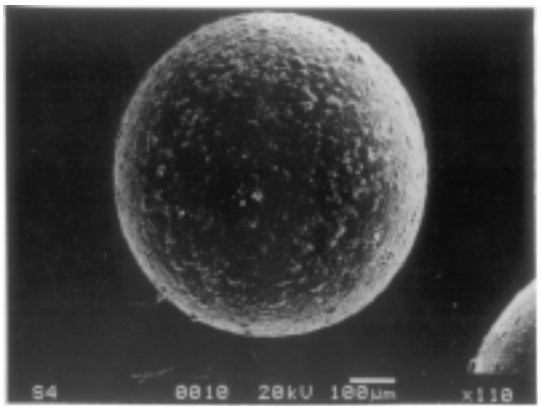


Fig. 1 : Scanning electron micrographs of drug loaded microsphere

The yields of microspheres were in the range of 76-91 % (Table 2). An increase in the amount of EC increased drug encapsulation efficiency. It is so because the higher the polymer to drug ratio, the higher probability of drug surrounded by polymer, which acted as a barrier to prevent from diffusion of drug into the external medium. The IR spectra of IS, EC loaded microspheres and blank EC microspheres are shown in Fig.2. Drug spectrum showed prominent peaks at 3277 cm-1, 3243cm-1, 2974cm-1,

Table 1: Effect of various parameters on mean particle size

Variable		Mean particle size (mm)
Drug-to- polymer ratio	1:1	324.59 ± 1.93
"	1:2	367.14 ± 4.94
"	1:3	383.55 ± 4.26
"	1:4	434.95 ± 0.36
Surfactant concentration	0.5%	257.97 ± 2.37
"	0.25%	434.95 ± 0.36
Stirring speed (rpm)	250	693.99 ± 13.32
"	500	434.94 ± 0.36
"	750	256.92 ± 2.74

*Standard conditions: drug -to-polymer ratio1: 4 surfactant concentration 0.25 %V/V;

Volume of processing medium 50 ml; stirring speed 500 rpm. Each observation is the mean (± SD) of three determinations

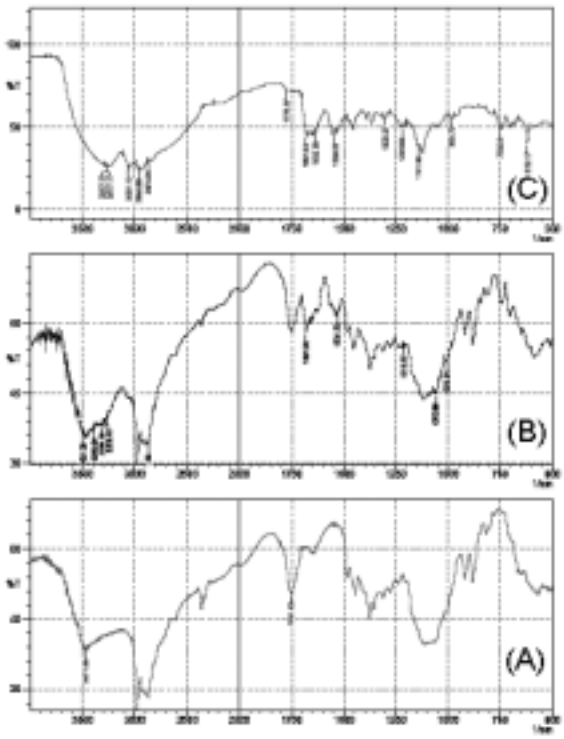
Table 2: Effect of drug to polymer ratio on yield and entrapment efficiency and various parameters of Korsemeyer Peppas model

Batch	Yield (%)	Entrapment efficiency (%)	r^2	n
F1	91.86 ±1.68	69.94 ± 2	0.998	0.41
F2	94.35 ± 2.40	82.80 ± 0.73	0.992	0.445
F3	84.88 ± 7.4	83.75 ± 1.15	0.996	0.476
F4	76.93 ± 2.52	90.08 ± 0.81	0.991	0.41

* r2 is the correlation coefficient and n is the release exponent of Korsemeyer Peppas model. Each observation is the mean (± SD) of three determinations

2874 cm-1 1681cm-1 and 1220 cm-1corresponding to OH stretching, NH stretching of secondary amine, aromatic C-H stretching, (-CH2-) stretching, C=O stretching and C-N stretching respectively. IS loaded microsphere exhibited peaks within the same region confirming the stable nature of drug during encapsulation process.

The release studies were carried out with 355 μm size fractions in order to keep the total surface area of the microspheres constant and thus, to get comparable results. Fig. 3 show that the release of IS from microspheres, illustrating the rate of drug release from the microspheres depended on the polymer concentration of the prepared devices. It was found that the release rate decrease with increasing the amount of the polymer. This can be explained by a decreased amount of drug present close to the surface and also by the fact that the amount of uncoated drug decreases with higher polymer concentration. The effect of particle size on the drug release was also studied, by using microspheres of different size fractions of 250, 355 and 500 μm, as shown in Figure 4. The release profile was in line with the general hypothesis of the effect of the particle size on dissolution[5-6]. As the particle size decreased, the drug release was fast because of more available surface area. In order to determine the mechanism



Formulation F4 (B) and Indinavir Sulfate (C)

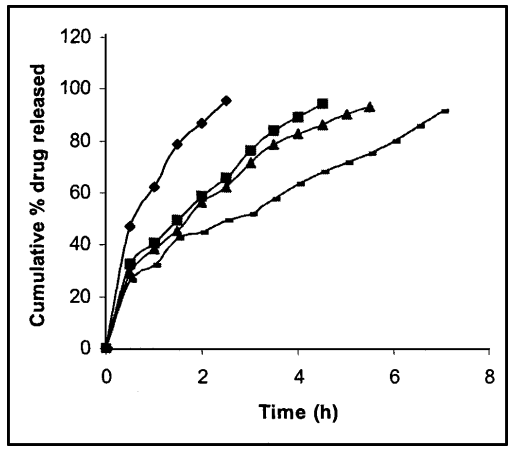


Fig. 3 : Cumulative percentage release of Indinavir (n = 3) from ethyl cellulose microspheres prepared with different drug to polymer ratio. F1 (—◆—), F2 (—■—), F3 (—▲—), F4 (—●—).

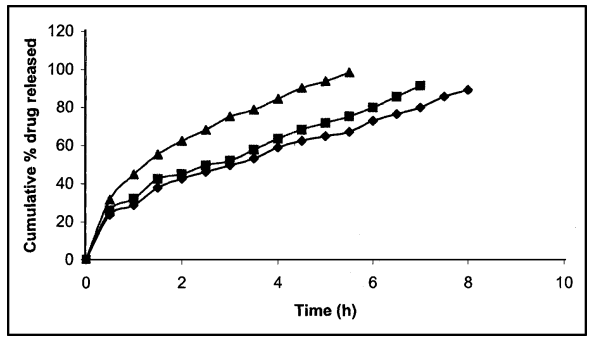


Fig. 4 : Effect of particle size on release profile of Indinavir loaded microsphere of F4. Release of drug from particle size fraction 250 µm (—◆—), 355 µm (—■—) and 500 µm (—▲—).

of drug release the data obtained were fitted in to Korsemeyer Peppas model so as to find out 'n' value, which describes the drug release mechanism[8]. The 'n' value of all formulations lies between 0 and 0.5 (Table 2) indicating the mechanism of the drug release to be diffusion controlled. The release also shows high correlation with Korsemeyer-Peppas model.

From the above study it could be concluded that a stable and sustained release formulation of anti HIV drug IS with EC can be prepared successfully by o/o emulsification solvent evaporation method, utilizing mixed solvent system consisting of acetonitrile and dichloromethane in a 1:1 ratio. The resulting microspheres were spherical, free flowing with high encapsulation efficiencies and the release was sustained up to 8 h (F4). The mechanism of the drug release from the microsphere was found to be diffusion controlled.

ACKNOWLEDGEMENT

The authors greatly acknowledge Macleods Pharmaceuticals Ltd., Mumbai, India for supply of Indinavir Sulfate as a gift sample. The authors are grateful to Indian Institute of Technology (IIT), Kharagpur, India, for help in performing characterization studies.

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FORMULATION AND EVALUATION OF NITROFURAZONE GEL CONTAINING COW'S GHEE

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Nagpur University, Nagpur[2]

ABSTRACT

The objective of the present work is to investigate the influence of cow's ghee on the release pattern of nitrofurazone. On the basis of previous research work three formulations namely:F-1,F-2 and F-3 were prepared. In the release studies carried out through dialysis membrane, using modified Franz diffusion cell, the release of drug was significantly influenced by ghee. During storage period no trace of rancidity was observed and the formulation was found to be stable from both pharmaceutical and aesthetic point of view.

Key words : Cow's ghee, Nitrofurazone, Interaction.

INTRODUCTION

The use of fats and oils as vehicles for cosmetics and drugs are being used dates back to the ancient Babylonians and Egyptians civilizations[1]. Bee's wax, spermaceti and wool fat are some of the well-known examples in India. Fat derived from the cow's milk commonly known as ghee has been used for this purpose.

In Ayurvedic system of medicine there are numerous reports on the formulation containing herbs and cow's ghee[2]. Ghee is a complex mixture of different saturated and unsaturated fatty acids including minerals, vitamins and other substances. Cow's ghee is believed to be a coolant, capable of increasing mental powers and physical appearance and curative of ulcers and eye-diseases. As a human food, ghee is considered to be superior to other fats[3].

The work carried out in our laboratory have shown excellent immunomodulatory, immunostimulant, sedative, anticonvulsant, memory enhancer, neuropharmacological, anti-inflammatory, antimicrobial, wound healing and a host of other such pharmacological activities in formulations containing ghee along with herbs[4-11]. The formulation containing ghee with neomycin or nitrofurazone exhibited synergistic wound healing activity, presumably because of antimicrobial activity of both the drug and fatty acids present in ghee, alongwith the cell tissue growth promoting activity of ghee[12,13].

Of further interest is the indication that cow's ghee accelerates tissue-building process. Under these circumstances it becomes logical to examine these hitherto unexplored aspect of therapeutic use of cow's ghee besides, offering excellent possibilities of its application in pharmaceutical systems. The objective of the present work was to formulate a topical preparation in the form of gel of ghee in combination with an antimicrobial drug, nitrofurazone and to find out whether ghee in any way influences the release of antimicrobial agent from its topical formulation. Nitrofurazone is a nitrofuran derivative and posses antibacterial action against a number of gram+ve and gram-ve bacteria[14,15]. Two ratios of drug: ghee was selected for final formulations on the basis of preliminary investigations using serial dilution method and other spectroscopic techniques. Present study was aimed to characterize and evaluate the formulated gel from release point of view and other pharmaceutical parameters.

MATERIALS

Nitrofurazone was obtained as gift sample from Glaxo-smithkline Ltd., Mumbai, India. Ghee was obtained as gift sample from Go-Vigyan Anusandhan Kendra, Nagpur, India and used as such. Carbopol 934F was procured from BF Goodrich, USA. Triethanolamine, butylated hydroxy toulene, benzalkonium chloride, propyl paraben were purchased from S.D.Fine Chemicals, Mumbai. Propylene glycol was obtained from E.Merck, Mumbai. Glycerin was purchased from Rankem, India.

METHODS

DESIGN OF BASIC FORMULATION

Dermatological gels (F-1, F-2 and F-3) were prepared as shown in Table 1. Carbopol-934F was added with stirring in distilled water and left to swell for 24 hrs. In order to avoid air entrapment triethanolamine was added drop wise to swelled carbopol with gentle stirring. Pre-equilibrated mixture of nitrofurazone and ghee in selected ratios, dissolved in ethanol was added to gel base followed by addition of the other constituents.

Table 1. Composition of different gel formulations.

Formulation	Composition	
Gel base	Carbopol-934	- 2.0%
	Triethanolamine	- 1.65%
	Propylene glycol	- 5.0%
	Glycerine	- 10%
	Benzalkonium chloride	- 0.5%
	Propyl paraben	- 0.02%
	Butylated hydroxy toluene	- 0.01%
	Water	- upto 100%
F-1	Nitrofurazone	- 0.2%
F-2	Nitrofurazone	- 0.2%
	Cow`s Ghee	- 0.14%
F-3	Nitrofurazone	- 0.2%
	Cow`s Ghee	- 0.3%

IN VITRO RELEASE STUDIES

Release behaviours of gels were measured using fabricated Franz type diffusion cell. The dialysis membrane (cellulose membrane, molecular weight cut off between 12000 to 14000, pore size 2.4nm) was presoaked in phosphate buffer of pH 7.0 for 24 hours. Presoaked membrane (cross-sectional area 3.14cm2) was mounted between the donor and receiver compartment of the diffusion cell and clamped into position. The prepared gel was uniformly spread over the membrane from the donor compartment side. The reservoir fluid (phosphate buffer pH 7.0) maintained in the receiver compartment at 37 ± 1^0C in the constant amount of 20ml, was kept in motion for 8 hours on magnetic stirrer at 500 rpm. Samples (1 ml) were withdrawn from the receiver compartment at regular intervals and replaced by the equivalent amount of thermostated fresh media. After adequate dilutions, the samples were analyzed at 375nm on UV-Spectrophotometer (UV-1600, Shimadzu, Japan). Experiments were performed in triplicate.

STABILITY EVALUATION

Since one of the objectives of the study was to develop a stable, spreadable and dispensable gel formulation, So, stability of the gel formulations was evaluated in terms of the changes in physical and chemical parameters, which were likely to effect the stability and acceptability of the formulations. Physical stability of gel formulations was evaluated in terms of physical changes like phase separation, changes in colour, odour, and consistency of the formulations.

Chemical stability was evaluated by determination of free fatty acid value, peroxide value, drug content uniformity and pH of developed formulation[15].

SPREADABILITY

A special apparatus was designed to study the spreadability of gel formulations[16].The spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability.

The spreadability was calculated by using the formula:

$$S = M .L/T$$

Where, S = spreadability, M = weight to upper slide, L= length of glass slide, T = time taken to separate the slides completely from each other.

EXTRUDABILITY STUDIES[17]

The formulations were filled in the collapsible tubes after the gels were set in the container. The extrudability of the formulation was determined in terms of weight in gms required to extrude a 0.5 cm ribbon of gel in 10 seconds.

RESULT AND DISCUSSION

Release of drug is a function of chemical and physicochemical properties of both the drug as well as that of a formulation. The diffusion of a drug across a membrane also depends upon the characteristics of the membrane. In the present study the release was measured in terms of its passage across the semi-permeable membrane.

The release of nitrofurazone from gels was significantly influenced in presence of ghee. The formulation, devoid of ghee showed 45 % release of nitrofurazone. In F-2 gel the release of drug was 34 % whereas, F-3 showed 58% drug release (Fig. 1). Previous studies have shown the existence of probability of interaction between nitrogen of nitrofurazone and carbonyl or hydroxyl group of fatty acid, resulting in some sort of complex formation[13]. This seems to be the major governing factor in alteration of drug release.

On the basis of pH, free fatty acid value and saponification value (Table 2), the formulation was

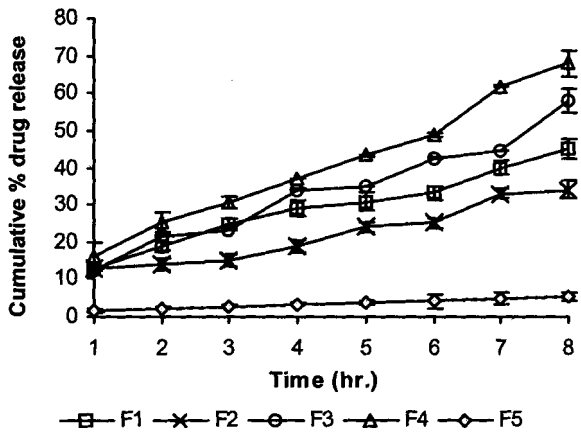

Fig.1 Comparative release of nitrofurazone from different formulations in eight hours.

Table 2. Physico-chemical characteristics of gel.

F	% Drug Content	pH	FFA	PV	SP	Consistency
F-1	94.90 (0.501)	5.81	1.35	1.7	5.42	Satisfactory
F-2	93.84 (0.956)	5.96	1.36	1.7	5.96	Satisfactory
F-3	92.67 (0.674)	5.73	1.85	1.85	5.76	Satisfactory
F-4 Oint. (Mkt.)	95.68 (1.02)	-	-	-	13.15	Oily
F-5 Cream (Mkt.)	92.36 (0.658)	-	-	-	11.30	Satisfactory

F= Formulation FFA= Free fatty acid value
PV=Peroxide value SP= Spreadability
Mkt.= Marketed

found to be stable without any traces of rancidity during six months storage period. Antioxidant incorporated in the gels might have prevented the development of rancidity.

The formulations were found to be satisfactory with respect to aesthetic values and pharmaceutical parameters such as extrudability and consistency.

CONCLUSION

Incorporation of cow's ghee results in alteration of drug release. This is dependent upon the quantity of ghee incorporated in the formulation. A slow release property may be helpful in doing away with the need of repeated dressings.

This study opens a novel possibility of formulation for topical application in which the release of nitrofurazone may be tailored to our desire by incorporation of cow's ghee in appropriate proportion.

ACKNOWLEDGEMENTS

The author* is thankful to UGC, New Delhi for financial support during the research work.

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ANLGESIA AND ANAESTHESIA DURING LABOUR : A BRIEF PHARMACOLOGICAL REVIEW

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Arya College of Pharmacy, Jaipur[2]

ABSTRACT

The use of medication during the labour period must be carefully assessed because they may potentiate the physiologic effects of pregnancy in the mother and may adversely affect the foetus. Almost every drug used for analgesia in the mother during labour crosses the placenta and can affect the foetus. Most analgesic agents are small in molecular weight (<1000), are lipid soluble, and have a higher pka, thus allowing for easy transit across the placenta. The use of new agents is investigational at this time, but holds promise for the future.

Almost every drug used for analgesia in the mother during labour crosses the placenta and can affect the foetus. The use of medications during the intrapartum period must be carefully assessed because they may potentiate the physiologic effects of pregnancy in the mother and may adversely affect the foetus. So it is important to carry out antopartum and intrapartum risk assessment to detect maternal and foetal complications before introducing other variables such as analgesia and anesthesia.

Most analgesic agents are small in molecular weight (<1000), are lipid soluble, and have a high pka, thus allowing for easy transit across the placenta. Narcotic agents, mainly meperidine, are the most common type of analgesic administered during pregnancy. In most cases, meperidines, given in 25 to 50 mg increments every 2 to 4 hours, is sufficient for pain relief during labour and has little effect on the baby other than decreasing the beat to beat variability of the fetal heart. At these dosage levels, if the foetus is depressed at birth, invariable it is not due to the analgesic. Although neuro behavioural changes have been associated with meperidine usage, these effects are self limited and have no long term consequences. Additionally, some have interdicted meperidine just before parturition because of the risk of fetal depression. However, most agree that the drug should not be withheld because the patient is going to deliver within in the next 1-2 hours.

Regional anesthesia is used more today in obstetrics than never before. The use of segmental epidural blocks has gained wide popularity in the United States. Epidural analgesia using amide derivative drugs (lidocaine and bupivacaine) may selectively block uterine pain during labour and has the advantage of also being able to render satisfactory anesthesia for delivery. Complications include inadvertent entry into the spinal canal and hypotension. Paresthesia, an infrequent complication, usually occurs with the ester based local anesthetics (2-chloroprocaine). Direct toxicity with high percentage bupivacaine (0.75%) has been observed after epidural anaesthesia. In addition, many Obstetricians believe that the use of conduction anesthesia prolongs labour and leads to an increased incidence of foetus in the transverse (vertex) position and the subsequent need for midforceps rotations or cesarean section. The adverse effects on the mother and foetus in this setting, however, are far outweighed by the benefits of this type of anesthesia.

Pyschoprophyllaxis (prepared child birth) has also gained wide acceptance in this country as a method of analgesia in labour and, if successful, is very rewarding for the parturient and her support

person. If unsuccessful, small doses of analgesic agent can be administered, and this permits the parturient to still actively participate in birth process. It is necessary to tailor psychoprophyllaxis to each patient, rather than to enforce a rigid protocol of anlgesia versus no analgesia, because hyperventilation with resultant hypocapnia and epinephrine release may result in fetal hypoxia directly related to natural child birth.

Sedatives and hypnotics are now used frequently in labour and delivery suites. Barbiturate usage, at least during the active intrapartum phase of labour, is almost entirely limited to the induction of general anesthesia for cesarean birth. For vaginal deliveries these agents are best avoided because they are ineffective as analgesic agents and there are disadvantages associated with their use in both mother and the foetus.

The use of new agents (such as endorphins or peridural morphine) are investigational at this time, but holds promising for the future. Other analgesic and anesthesia techniques include hypnosis and acupuncture. These techniques, although demonstrated effective in some studies, have not generally been accepted by the medical community. Additionally, patient control analgesia is becoming increasingly popular for labor analgesia.

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PHARMBIT

ISSN : 0973-6204

Scientific Journal of Pharmaceutical Society

Vol. XV No. 1 Jan-Jun, 2007

CONTENTS

Single copy INR 150

Printed & Published by :
Dr. R.N. Gupta, *Editor*
on behalf of Pharmaceutical Society
Department of Pharmaceutical Sciences
BIRLA INSTITUTE OF TECHNOLOGY
Mesra, Ranchi, India
Printed at Kailash Paper Conversion Pvt. Ltd.
2, Bharatpuri, Purulia Road, Ranchi-834001
Tel.: 0651-2532251, Fax : 0651-2532285

1034/06/07 • Kailash Paper, Tel.: 0651-2532251