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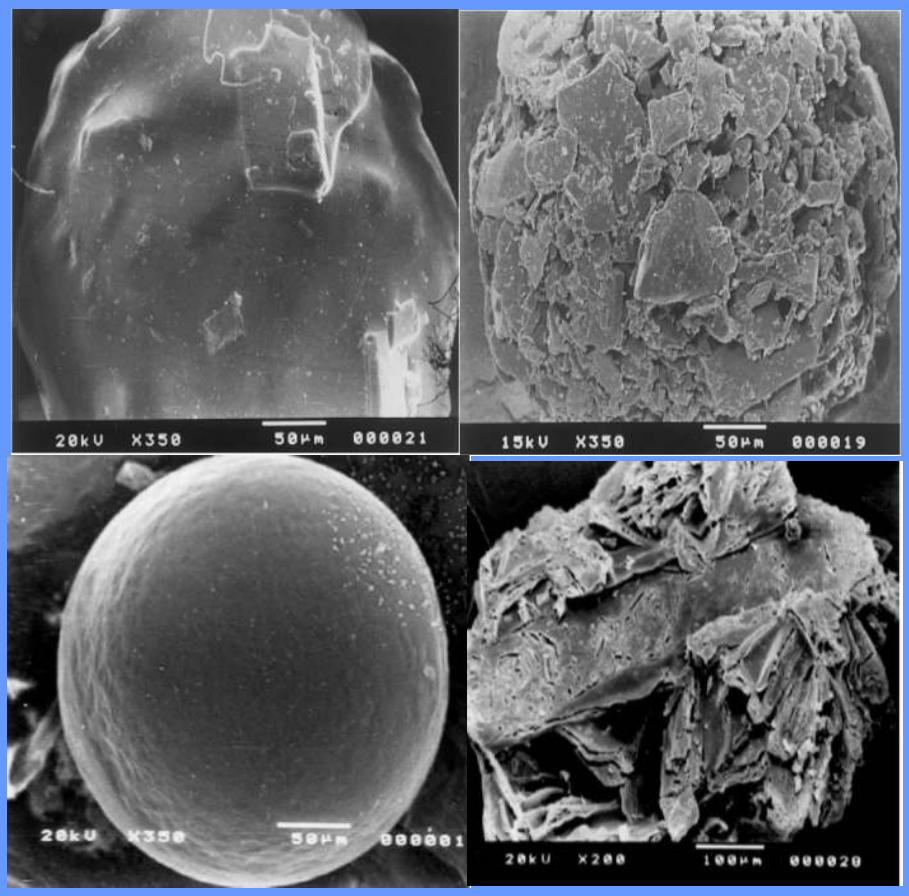

Xenoestrogens and Metainflammation: An interplay between Immune System, Metabolism and Obesity

Macroemulsions and Microparticles: Uncovered Mechanistic Insight and Non-conventional Application Potential

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The cover page contains a figure from the article of Dr. Tamilvanan Shunmugaperumal

EDITORIAL

Drug discovery is a challenging task, only a few venture in this direction. The profit margins are small, the recognition for work is rare, the success percentage is relatively small, in comparison to other sciences / technologies. Yet the requirement of drugs is ever increasing. Motivating scientists to take up pharmaceutical research is an important activity. The Department of Pharmaceuticals (DoP), Ministry of Chemicals and Fertilizers has taken up this task and successfully executing the same. Its efforts in promoting the fundamental as well as applied pharmaceutical sciences are increasing year-on-year. Publishing CRIPS is one such activity. Encouraging students to take up research is a pioneering effort of DoP. Recent advancement in this direction is in the form of increasing number of fellowships and increasing the fellowship amount.

The interplay between immune system metabolism and obesity is one of the poorly understood phenomenon in pharmacology. Out of the several efforts, xenoestrogens and metainflammation studies are pioneering efforts. In issue, Gena and coworkers explained the details of the research efforts in these topics. Normal metabolism in cells is disturbed by xenoestrogens. Macrophages surrounding adipocytes get activated during the onset of obesity. In this review, the roles of xenoestrogens in causing metainflammation, the connecting link between metainflammation and the immune system, the molecular basis of obesity, metabolic considerations in these topics have been explained in detail.

The article by Shanmugaperumal and coworkers discussed uncovered mechanistic insights into macroemulsions and microparticles. They proposed many non-conventional applications of these systems. In the abstract, the objectives of the review were elaborated. It was considered that the micron-level sizing is less-expensive and it is worth switching back to their production. The cases studies considered include – (i) indomethacin-ibuprofen loded polystyrene (ii) ornidazole loaded PEG. Methods of microemulsion formulations, stability analysis and their therapeutic potential are also included in this article.

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Review Article

Xenoestrogens and Metainflammation: An interplay between Immune System, Metabolism and Obesity

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Obesity is the greatest threat to mankind in twenty first century. It is accompanied with low grade chronic inflammation (metainflammation) which persists in all the tissues involved in energy balance. Obesogens such as xenoestrogens enter into the body and disrupt normal metabolism of cells which are responsible for fat deposition namely adipocytes. The research findings demonstrated that macrophages play a crucial role in metabolic tissues during the onset of obesity. When an intricate balance between metabolism and innate immunity is disturbed, macrophages surrounding adipocytes get activated and converted into pro-inflammatory subtypes. Pro-inflammatory macrophages secret pro-inflammatory cytokines such as Interleukin 6, Tumor Necrosis Factor-α and C- reactive protein. In the present review, an attempt has been made to elucidate the molecular mechanisms involved in xenoestrogen-induced obesity and metainflammation. Further the role of immune system and the involvement of metabolism in the genesis of metainflammation have also been explored briefly.

Introduction

According to the World Heart Federation (WHF) report of 2022, approximately 2.3 billion individuals were suffering due to obesity worldwide. Interestingly, the number of obese people is higher as compared to the underweight in almost every part of the world. As per the currently ongoing trend, approximately 2.7 billion adults i.e. around 33% of the total global population are supposed to be obese by the year 2025.[1] Along with obesity, the cases of other metabolic diseases like type 2 diabetes mellitus and non-alcoholic fatty liver disease (NAFLD) are also rising significantly.[2,3] Therefore, scientists are exploring to find out the intricate relationship among different metabolic diseases as well as to understand the pattern of disease initiation and subsequent complications. In order to decipher this relationship, the research in the last decades has been oriented towards the concept of immunometabolism, an interface between metabolism and immune system. Apparently, it seems that nutrient- (metabolic process) and pathogen-sensing (immune system) systems are working in a mutually exclusive manner in higher organisms. In lower invertebrates such as drosophila, these two systems are not only working intricately but also are located intertwiningly, known as the fat body.[4] The close association between these two consistently working systems gave birth to a new concept called "metainflammation", the term which was first coined by Hotamisligil in year 2006.[5] In most of obese people, the proliferating adipocytes initiate a cascade of low grade systemic inflammation known as metainflammation. If this persists for a longer period, it can propagate deleterious effects to the metabolically active organs such as pancreas, liver and heart.[6,3]

It is undeniable that unhealthy dietary practices and a sedentary lifestyle have been considered as the predominant contributing factors in continuously surging obesity cases. Moreover, an emerging body of scientific literature emphasizes that exposure to different environmental chemicals act in tandem with these factors to exacerbate the incidence of obesity at an alarming degree.[7] For instance, xenoestrogens, a group of chemicals which mimic the natural estrogen synthesized in the body, play a pivotal role in the development of obesity, other metabolic diseases and metainflammation.[8,9] Among the xenoestrogens, BPA is the most abundant and most studied chemical in both humans and experimental

Keywords: Metainflammation, Obesity, Xenoestrogen, Bisphenol A, Macrophage, Immunometabolism

models.[10,11] Various *in-vivo* studies have shown that pre- and post-natal exposure to Bisphenol A (BPA) increased the body weight of pups and induced permanent obesity in the adult rodents.[12-14] Therefore, it is clear that these xenoestrogens can target the early phases of life leading to the lifelong effects. Being non-biodegradable and highly lipophilic in nature, the xenoestrogens tend to accumulate in the adipose tissue and cause inflammation for a longer period.[15] In the present article, an attempt has been made to provide a critical insight regarding the role of metainflammation in xenoestrogen-induced obesity. Further, the molecular aspects of the existing interface between xenoestrogens, immune system and genesis of metainflammation has also been explored briefly.

Role of Xenoestrogens in Obesity

Xenoestrogen can cause obesity mainly through metabolic disruption.[16,17] The obesogenic actions of BPA have been extensively studied in the *in-vitro* and *in-vivo* models.[16] Ultimately, the mechanisms through which these xenoestrogens exert their metabolic disruption and obesogenic effect, even when the level of exposure is below the No Observed Adverse Effect Level (NOAEL), are being elucidated by different scientific studies. The relationship between the exposure level of xenoestrogens and weight gain forms an inverted "U" shape, curve where the lower exposure level exerts more deleterious effect than the higher exposure level.[18] Pro-opiomelanocortin (POMC) neurons in the hypothalamus restrict food intake while Agouti-Related Peptide (AgRP) and Neuropeptide Y (NPY) do the reverse effect. The experimental *in-vivo* models showed that BPA-exposed mice had POMC neuron innervations and the AgRP and NPY peptides activities were abolished.[19,20] Apart from doing dysbalance in neuronal control, xenoestrogens can alter the carbohydrate metabolism by causing insulin resistance and necrosis of pancreatic β-cells.[21] They also increase the number as well as the size of white adipocytes by elevating triglyceride content, lipoprotein lipase activity and adipogenic transcription factor expression like CCAAT Enhancer Binding Protein-ß (C/EBP-ß).[7,22] Thus, xenoestrogens perturb the intricate balance between the neuronal control and metabolism of the body and cause excess weight gain as well as obesity.[16]

Xenoestrogens as a Causative Factor for Metainflammation

Xenoestrogens are compounds which have an ability to interfere with the natural hormones which are accountable for the regulation of various physiological functions such as development, behavior, fertility as well as maintaining the homeostasis in the body.[23] The matter of concern is that there are several xenoestrogens which are lipophilic in nature. As Body Mass Index (BMI) increases, these lipophilic molecules accumulate in the adipose tissue. They are non-biodegradable; therefore their concentration tends to increase with time and further continuous exposure is a subject of concern.[24] Basically, there are two types of xenoestrogens: natural (phytoestrogens) and synthetic[23], however some authors do not consider natural ones in the list of xenoestrogens.[25,26] Among the synthetic xenoestrogens, BPA is the most abundant in use; whereas isoflavones form the most important group of phytoestrogens.[27] Although, isoflavones exhibit antioxidant, anticancer, antimicrobial and anti-inflammatory properties, some of them like soy isoflavones are also reported to cause obesity and other metabolic disorders.[28,29] Both of them imitate the action of estrogen and alter the metabolic processes in the body. These are capable to upregulate different transcription factors like Peroxisome Proliferator-Activated Receptor (PPARγ), C/EBP and Nuclear Factor Erythroid 2-related Factor 2 (Nrf2), which can ultimately lead to the induction of obesity and associated metainflammation.[14] Table 1 depicts some of the important xenoestrogens, their sources along with their role in obesity and metainflammation.

Many of xenoestrogens cause metainflammation in a similar way like BPA does. BPA significantly increased the expression of several genes involved in adipogenesis and lipid accumulation, including C/EBPα, C/EBPβ, PPARγ, Fatty Acid Synthetase (FASN) and Sterol Regulatory Element Binding Protein1c (SREBP1c). The up-regulation of these adipogenic transcription factors and enzymes are reported to be involved in the development of metainflammation.[30-32] BPA has an ability to interact with Nuclear Receptors (NR) including Retinoid X Receptor (RXR), PPARγ, Estrogen Receptors (ER), Thyroid Receptors (TR) and Glucocorticoid Receptors (GR), as a result of which it can induce differentiation of adipocytes and lipid accumulation.[33] BPA can also activate classical transduction pathways of ERα and ERβ, which can further reduce adiponectin secretion and increase the proliferation of adipocytes.[34] Organochlorines like Dichlorodiphenyltrichloroethane (DDT) and endosulfan increase the expression of Aryl Hydrocarbon Receptor (AHR) transcription factor, which has the potential to elevate the production of the aromatase, a CYP450 mediated enzyme.[35,36] Aromatase, in turn, converts androgen to estrogen which upregulates the PPAR and c/EBP transcription factors.[36] PPAR and ER family has been reported to correlate the different molecular

pathways involved in the process of metainflammation.[5] Males are more prone to metainflammation and related metabolic diseases as compared to age-matched pre-menopausal females.[37] This type of difference in susceptibility is due to the presence of estrogen, which plays a protective role in pre-menopausal females. Further, post-menopausal females develop more metabolic disorders than age-matched males.[38]

Metainflammation and Immune System: The Connecting Link

Among the immune cells, macrophages significantly contribute to the obesity-induced systemic inflammation. Macrophages have an ability to quickly sense their microenvironment and change their metabolic profile as well as express a wide variety of inflammatory markers.[44] Under the stressful conditions, macrophages are polarized into pro-inflammatory macrophages (M1 subtype), which mainly secretes pro-inflammatory cytokines. Once the stress is over, another type of macrophages (M2 subtype) get activated and play a crucial role in tissue repairing.[45] Interestingly, M2 macrophages utilize fatty acids as the energy source and produce ATP through β-oxidation and oxidative phosphorylation, which is more time consuming. Conversely, M1 macrophages quickly undergo aerobic glycolysis.[46] Macrophages during metainflammation reportedly behave like M1 macrophages to a great extent.[47] These metabolically activated macrophages express low levels of CD206 (overexpressed in macrophages of non-obese animals) and elevated the levels of CD11c, CD36, Macrophage Scavenger Receptor 1 (MSR1), ATP-binding cassette A1 (ABCA1), adipose differentiation-related protein such as Perilipin-2.[48,46,49] Various types of immune cells in the adipose tissue can affect the shift in macrophage polarization. For instance, neutrophils induce this change by using protease elastase, T-lymphocytes by using interferon-γ, natural killer cells induce polarization by TNF-α and MCP1 and B cells contribute by producing IgG antibodies.[50,47] Macrophages expressing CD11c have been linked to insulin resistance and are located in the crown-like structures, which encircle necrotic adipocytes to eliminate them through a process called exophagy.[46] This process results in the uptake of FFA and lipids by macrophages and the formation of foam cells.[51] In summary, obesity induces some changes in the phenotype and behaviour of macrophages which contribute greatly to the overactivation of innate immunity system of our body leading to the initiation of low grade systemic inflammation.

Obesity and Metainflammation: The Molecular Basis

Lipids are involved in the coordinated regulation of metabolic, inflammatory and innate immune processes. The quest for elucidating the molecular signaling involved in metainflammation started around a decade ago when researchers discovered high levels of Tumor Necrosis Factor-α (TNF-α) in the adipose tissue of obese mice. This finding established a clear connection between obesity and chronic inflammation in the experimental mice model.[52] Later, it was established that TNF-α is not released by adipocytes but rather by the macrophages which surround them.[53] TNF-α is a marker of local as well as systemic inflammation, therefore high chances of crosstalk existed between adipocytes and immune system under obese condition.[54,55] According to literature, Endoplasmic Reticulum (ER) can serve as a common target and in fact, it is considered that ER begins the inflammatory cascade in the metainflammation process.[5,56]

In obesity, the elevated levels of Free Fatty Acid (FFA) in the adipocytes increase ER stress which can further activate a number of inter-connected pathways.[57] FFA stimulates the unfolded protein response which is mediated by nutrient fluctuations, hypoxia and the presence of different toxins. This, in turn, generates additional molecular targets such as Activating Transcription Factor 6 (ATF-6), Inositol-Requiring Enzyme Type 1 (IRE1) and Protein Kinase R-like Endoplasmic Reticulum Kinase (PERK), which can further lead to metainflammation.[58,59] It has been reported that ATF-6 increases stress to Golgi body, IRE1 stimulates the lipid droplet formation and PERK binds with PPAR and C/EBP proteins.[60] These three phenomena can increase the oxidative stress in the system and activate Jun N-terminal Kinase (JNK) and IκB Kinase Complex (IKK) factors to cause inflammation and necrosis of adipocytes.[61] Additionally, FFA directly upregulates the expression of PPAR, C/EBP and Nrf2 as depicted in the figure 1. All of these mediators alter the expression of IL-6 and TNF-α which can further stimulate monocyte to macrophage activation. The macrophages also produce IL-6 and TNF-α which also help to initiate the vicious cycle of inflammation.[59]

The leakage of calcium (Ca^{2+}) ions from the outer membrane of ER is another phenomenon which takes place under ER stress. The excess of Ca^{2+} ions are responsible for the mitochondrial damage and secretion of cytochrome-c. This further leads to apoptosis by binding with the Apoptotic Protease Activating Factor-1 (Apaf-1).[62] Both ER stress and mitochondrial damage increase the Reactive Oxygen

Species (ROS) levels which in turn activate JNK and IKK. Both JNK and IKK up-regulate the Monocyte Chemoattractant Protein-1 (MCP-1), IL6 and TNF-α expression but decrease the production of adiponectin, a cytokine produced exclusively by the adipocytes.[63] Reduction in the adiponectin level is also accomplished by GPR30, a G-protein coupled receptor involved in the production of anti-inflammatory cytokines like IL10. Estrogen can have a rapid non-genomic response via GPR30. The GPR30 knockout mice have reportedly exhibited elevated levels of pro-inflammatory cytokines and low adiponectin levels in their circulation.[64] The reduced production of adiponectin and increased production of pro-inflammatory cytokines block the action of Insulin Receptor Substrate (IRS), resulting in the insulin resistance in adipocytes. Insulin receptor via mTORC1-Egr1-ATGL pathway ameliorates the degradation of triglyceride into FFA which increases the size of lipid droplet.[65] Due to large lipid droplets, ER tends to synthesize more proteins to package the enlarged lipid droplets and this phenomenon is responsible for the excessive ER stress.[66] From the existing literature, it is clearly evident that the pathophysiology of metainflammation relies on a complex intertwined pathways involving the metabolic and immune system, which start with ER stress and then progress to other cell-organelles leading to the production of pro-inflammatory cytokines. The purinergic system, specifically the metabolites ATP and adenosine, plays a significant role in the development of metainflammation.[67] Adenosine exhibits anti-inflammatory properties by inhibiting Th1-polarizing responses and promoting the production of anti-inflammatory cytokines and Th2-polarizing responses. On the other hand, ATP, particularly at high extracellular concentrations, contributes to inflammation and cell death.[68,69] In metainflammation, there is a decrease in adenosine levels and a significant increase in ATP levels.[69]

Metabolic Considerations in Metainflammation: Role of Pancreas and Liver

In the context of metainflammation, the pancreas and liver are primarily affected. In the pancreas, metainflammation is associated with two main inflammatory pathways, JNK-AP-1 and IKK-NF-κB, which are connected to IRE-1 and PERK activity during ER stress.[70] These pathways involve interactions between IRE-1 and JNK activation through TNF receptor-associated factor 2 (TRAF2), as well as the association of IRE-1 and PERK activation with the IKK-NF-κB pathway. The activation of IRE-1 and PERK are also associated with the IKK-NF-κB pathway, but through distinct mechanisms. IRE-1 interacts with IKK-ß through TRAF2, whereas PERK activation leads to the degradation of IκB, thereby facilitating NF-κB activity.[71,72] In the liver, metainflammation occurs due to the entry of excessive amounts of free fatty acids (FFA) from necrotic adipose tissue, leading to lipid accumulation in hepatocytes and induce lipotoxicity.[73,74] The liver, like adipose tissue, has resident macrophages called Kupffer cells, and the interaction between hepatocytes and Kupffer cells follows a similar pattern as in adipose tissue. As the liver is crucial for carbohydrate and fat metabolism and relies on insulin, the excess lipid accumulation results in insulin resistance and the production of pro-inflammatory cytokines, disrupting the metabolic regulation.[75,76]

Future perspectives

The purinergic system is a crucial modulator of metainflammation and its role has been investigated in the aetiology of osteoarthritis.[67] The exploration of the role of purinergic system mediated metainflammation in metabolic disorders might open up a new therapeutic avenue for the disease. The relationship between metabolism and inflammation via epigenetic regulation of gene expression is another area which needs more research and may eventually lead to a potential clinical intervention strategy. Recent research suggests that lysine acetylation of both histone and non-histone proteins cause alterations in energy metabolism during chronic inflammation.[77] Therefore, by regulating the expression of pro- and anti-inflammatory mediators, deacetylase inhibitors or activators may be further strategies to prevent macrophage-induced metainflammation. Despite the significant progress made over the past few decades, many concerns regarding the processes of macrophage polarisation and metainflammation remains unanswered. The interaction between macrophages and their milieu is a complicated and dynamic process due to the heterogeneous and versatile character of macrophages. At present the understanding of these interactions in *in-vivo* conditions are limited. These issues can be addressed through the incorporation of new technology, such as computational biological methods for better understanding and interpretation.

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Review Article

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Macroemulsions and Microparticles: Uncovered Mechanistic Insight and Non-conventional Application Potential

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The objectives of this review are (1) to provide the rationale behind choosing micron-level sizing particulate systems like macroemulsions and microparticles, (2) to recollect the presence of two different forms of drug molecules inside the microparticles and to show the utility of eutectic forming excipients for making microcapsules from hydrophilic polymer-based micron-level particles, (3) to present non-conventional application potential of hydrophilic polymer-based micron-level particles and (4) to portray the dispersed oil droplets having a bicompartmental structure in oil-in-water (o/w) macroemulsions along with their possible utility for accommodating multiple cargoes. The potential of making micron sized particles rather than nanometer sized particulate systems is exemplified in a few case studies. The bicompartmental architecture observed in dispersed oil droplets of o/w macroemulsions is a welcome new addition/contribution to emulsion science. This review, therefore, explores the uncovered mechanistic insight and non-conventional application potential related respectively to macroemulsions and microparticles in a non-comprehensive manner.

Introduction

Solid oral dosage forms may be presented as either single units or multiple units. Single units can include soluble and insoluble matrix tablets, coated tablets, or capsules. Multiple units are generally presented as active pharmaceutical ingredients (APIs) (microparticles) or API-loaded beads contained within an outer unit such as a capsule. However, the presentation of the API in multiple unit dosage form provided the following advantages over single unit tablet or capsule dosage form as evidenced by the reported works of different research groups. Multiple unit dosage form for oral use, modifying the dissolution of the API, allows the administration of much smaller API amounts than single unit doses and provides a method of releasing the active ingredients at the desired rate.[1] Multiunit microparticulate dosage forms pass through the gastrointestinal tract avoiding the vagaries of gastric emptying and different transit rates, and, thereby, release APIs more uniformly.[2] Multiple unit system spreads in a large area of absorbing mucosa and prevents exposure to high API concentration when compared to single unit dosage forms on chronic dosing.[3] Compared with single unit dosage forms, multi-particulate oral API delivery systems reduce the risk of systemic toxicity due to dose dumping and local irritation, and ensure predictable gastric emptying.[4] On the other hand, oral colloidal dispersions having macron-or nano-sized dispersed particles encapsulated with a single API molecule can also be made. Being nanosized particles, a lot of advantages such as increased API solubilization, API targeting possibility, multifunctional activity creation, minimizing API toxicity, etc., are evidently being achieved.[5-7]

Unlikely long-or short-term stability problems and thus the potential deprival in the positioning of successfully marketed products are the two major concerns of formulation scientists who involve in

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the development of API-loaded nanometer-sized delivery systems. The examples of such delivery systems include cyclodextrin-based complexes, micelles, nanospheres including nanocapsules and nanoparticles, nanoballoons, nanosized emulsion, liposomes, etc. The nanometer-sized delivery systems are routinely being manufactured by the utilization of somewhat sophisticated size-reduction machinery like homogenizers, microfluidizer and ultrasonicators. Moreover, the addition of specialized multifunctional excipients such as anionic and cationic lipids, liquid and solid polyethylene glycols, nonionic surfactants, natural and synthetic biodegradable polymers, polyoxyethylene group containing synthetic co-polymeric molecules, etc., are necessary to make the delivery systems. Despite the involvement of both multifunctional excipients and sophisticated machinery, the produced so-called nanometer-sized delivery systems are still posing challenges for formulation scientists. The challenges are in terms of desired stability during their production stage and the finished product's self-storage time until their administration into patients. Moreover, each one of the nanometer-sized delivery systems is articulated with special/unique stability-related issues, and these issues are generally overcome by the addition of additional excipient molecules into those systems. For example, the therapeutic oil-in-water (o/w) nanosized emulsions are manufactured by the inclusion of multiple emulsifier molecules. The emulsifiers are usually possessing diverse affinity characteristics: one emulsifier contains aqueous affinity, another one consists of oil affinity and a third one possesses amphipathic/amphoteric characteristics. Whatever the characteristics, the selected emulsifier molecules need to localize at the oil-water interface of the emulsion for stabilizing the dispersed oil droplets against droplet-droplet collision, droplet coalescence, etc. Likewise, more or less similar to the therapeutic nanosized emulsions, the nanocapsules systems are also unequivocally being manufactured by using natural, semi-synthetic and synthetic biodegradable polymers along with oil core and multiple emulsifier molecules. To reduce the auto-oxidation problems associated with oil-, lipid-or nonionic surfactant-based nanometer-sized delivery systems, the antioxidant molecules (either a single or even multiple compounds) such as ascorbic acid, alpha-tocopherol, etc., are purposefully incorporated into these systems as additional excipients. One more popular excipient usually added into the nanometer-sized delivery systems in the earlier time is the antimicrobial agent or preservative. However, its usage is heavily dependent on the route of application of the developed API delivery systems. The use of antimicrobial agents or preservatives is currently restricted for topically applied nanometer-sized drug delivery systems especially if they are meant for ocular use. Taking the issues/problems of long-or short time stability associated with nanometer-sized drug delivery systems into consideration, the formulation scientists look back into the formulation development stage wherein the penultimate step of the nanometer-sized drug delivery systems involved in the production of particles sizes that ranged in the micron-level sizing. In general, the nanometer-level sizing always believes to produce a meta-stable form of particles, and the particles thus produced (due to the involvement of both multifunctional excipients and high-energy size-reduction machinery) are indeed tried to unit together at the expense of smaller (nanometer)-sized particles. In the end, the meta-stable nanometer-sized particles over the time period are slowly converted into stable particles having micron-level sizing. Moreover, this auto-conversion process particularly occurs especially if the surrounding continuous/suspending medium is an aqueous core. The inherent, time-dependent auto-conversion process that occurs within the developed nanometer-sized drug delivery systems thus gives an impetus for formulation scientists to go back (and look) into therapeutic potentials of particulate systems having the stable micron-level sizing particles that are possibly being produced (with the help of low-energy size-reduction machinery) at the particulate production stage itself.

The selected and further discussed micron-level sizing particulate systems in this review include macroemulsions and microparticles. The objectives of the current review are (1) to provide the rationale behind choosing micron-level sizing particulate systems like macroemulsions and microparticles, (2) to recollect the presence of two-different forms of drug molecules inside the microparticles and to show the utility of eutectic forming excipients for making microcapsules from hydrophilic polymer-based micron-level particles, (3) to present non-conventional application potential of hydrophilic polymer-based micron-level particles and (4) to portray the dispersed oil droplets having a bicompartmental structure in o/w macroemulsions along with their possible utility for accommodating multiple cargoes. This review starts with a brief discussion of the rationale for choosing a micron-level sizing particulate system.

Rationale behind choosing micron-level sizing particulate system

The long-or short time stability studies primarily conducted at lab-scale on the micron-and nano-levels sizing particulate systems indicate that the micron-level sizing particulate systems possess somewhat better withstanding capability in terms of both dynamic and kinetic aspects in comparison to the nano-level sizing particulate systems. The complete or partial elimination of a high level of energy input from external size-reduction machinery and possible omission of additional multifunctional excipients incorporation into the drug delivery systems make the micron-level sizing particulate systems more attractive in the sense of lesser manufacturing expenses than the manufacturing expenses associated with the production of nano-level sizing particulate systems which eventually obtained with the help (or sometimes advantage) of both the external size-reduction machinery and additional multifunctional excipients. On the other hand, the clinical or therapeutic efficacies of nano-level sizing particulate systems are well-established through both research publications and the successful positioning of a few commercial products. Unfortunately, the clinical or therapeutic efficacies associated with the micron-level sizing particulate systems are not so well-established and the comparative clinical or therapeutic efficacies study between these two (micro vs. nano level) particulate systems are never the focus of research publications. In other words, the potentiality of nano-level sizing particulate systems is constantly achieved just by comparing them with their drug powder or solution counterparts. No effort or complete ignorance is the case always pending at the researchers' side in terms of comparative clinical or therapeutic (efficacy) experimental studies between micron-and nano-levels sizing particulate systems. The constant/rapid evolution in inter-disciplinary scientific research works results in many outcomes in terms of publications, patents and commercialization which are totally in favor of the nano-level sizing particulate systems that too, are based merely on comparative evaluation between the nano-level sizing particulate systems and their drug powder or solution counterparts. But in terms of possible reduction in the production cost, improved final product stability and consumer affordability, it is the right junction to ask/put the question, "Why we aren't switching back to the production of less-expensive micron-level sizing particulate systems, if at all, these products are able to show rigorously the clinical or therapeutic efficacies that are similar or slightly lower or better compared to the clinical or therapeutic efficacies afforded by the nano-level sizing particulate systems for the management or treatment of particular syndrome". The switching-back process additionally renders the advantages of producing less-expensive products without compromising the clinical or therapeutic efficacies for the management or treatment of a particular syndrome. This review will try to cover the experimental work performed at a laboratory scale using the micron-level sizing particulate systems (macroemulsions and microparticles) for the management of a few symptoms like eradication of inflammation causing microbes in chronic periodontal disease or Acne vulgaris disease condition and acute or chronic pain associated with the primary dysmenorrheal condition.

Uncovered mechanistic insight and non-conventional application potential of microspheres/microparticles

When the solid APIs are incorporated into matrix/particulate forming hydrophobic or hydrophilic polymer and molten oil or lipid excipients, there are two modifications will occur on the physical states of solid APIs: molecular dispersion/solution and simple drug crystal formation within the developed matrix/particulate structure. If the molecular dispersion/solution formation occurs between the API and matrix/particulate forming excipients, then, the API is likely to be released rapidly from the matrix/particulate system at the time of dissolution test. The matrix/particulate structure also appears as smooth-surfaced when observed using electron (scanning or transmission) microscopic techniques. Conversely, the occurrence of drug micro-or nano-crystal formation within the matrix/particulate structure results in the retardation of drug release at the dissolution testing time and further leads to the creation of rough-surfaced structure on the developed matrix/particulate system when visualized under these two electron microscopic techniques. Irrespective of the physical forms of the final products (either liquid-retentive or solid free-flowing), the generated matrix/particulate structure shows the presence of either the molecular dispersion/solution or the drug micro-or nano-crystal within it and this structural discrimination is all depending on the APIs physicochemical properties, matrix/particulate forming excipients selected and manufacturing process conditions/parameters. For instance, the API is in dissolved form within the

polymer solution and the microencapsulation used is a spray drying technique, then, the final microsphere structure is of matrix or monolithic (or molecular or solid solution) type. Conversely, if the API is in dispersed form within the polymer solution and the same spray drying technique is employed, then, the final product belongs to the drug reservoir (sometimes called microcapsules) type wherein the API molecules are simply embedded in a well-defined polymeric wall structure.

At the very outset, the microparticles are multiple-unit drug delivery systems intended primarily for the oral route of administration. Later on, the parenteral injections are developed based on microparticles prepared from biodegradable polymers. The main functions of the microparticles for oral drug delivery are to avail taste-masking of bitter or salty APIs and to obtain sustained drug release of APIs for reducing the dosing frequency. For parenteral injectable microparticles, the main advantage is to control the API release over the time periods of days, weeks, months or even years. The controlled/ sustained release of incorporated API from the microparticles is one of the main reasons why the microparticles are generated for both oral and parenteral uses. Concerning the utility of microparticles for topical (percutaneous and buccal cavity) administration, it becomes necessary to incorporate the generated hydrophobic and/or hydrophilic polymers-based microparticles into a semi-solid carrier system, called gels or ointment base, so that the solid free flowing microparticulate structure will reside inside the semi-solid base when applied topically onto the patient's skin or teeth surfaces. Whatever it may be the routes (oral, parenteral and topical) of administration and the types of polymers (hydrophobic and/or hydrophilic) used, the internal structure has a profound influence on the final performances of drug-laden microparticles at both in vitro and in vivo conditions. The internal structure here indicates simply the presence of API either in molecular dispersion/ solution state or in micro-or nano-crystal state inside the generated hydrophobic and/or hydrophilic-based microparticles. Again, the above-said two different states of API within the microparticle structure rely heavily on the API's initial loading into the microparticles. The consequences due to the presence of drug molecular dispersion/solution and micro-or nano-crystal within the microparticles are exemplified below in two different case studies.

Case study-1: Indomethacin and Ibuprofen-loaded polystyrene microparticles

Following oral ingestion, the controlled/sustained release single-unit dosage forms such as tablets or capsules keep their integrity starting from the stomach and small intestine to the large intestine. These single-unit dosage forms also show variations in gastric emptying time value and thus the diversified transit rate at different parts of the gastrointestinal tract. Moreover, these single-unit systems always associate with the risk of dose dumping leading to local and systemic side effects due to the sudden rise in drug concentrations. On the other hand, multiple-unit dosage forms like matrix (microspheres, microparticles) or reservoir (microcapsules) and coated beads are quite free from these problems. Because after administration, they pass through the gastrointestinal tract smoothly and uniformly like a solution that results in a uniform drug release from the multiple-unit dosage forms and thus ensures the absorption of the drug throughout the gastrointestinal tract.[2-4] They also reduce any irritation or local side effects caused by local high concentrations of drugs. Matrix systems (microspheres, microparticles) are selected rather than reservoir systems (microcapsules) and coated beads because of the ease of manufacture and because of the relatively high loading doses required. To make oral microparticulate dosage forms for nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and ibuprofen, the biocompatible and hydrophobic polymer, polystyrene was selected. For a poorly soluble drug like ibuprofen, the relatively high-loading doses usually lead to bulky dosage forms, particularly with respect to reservoir systems.

The internal structures of these two developed microparticles loaded with indomethacin and ibuprofen were mechanistically analyzed as a function of drug loadings using scanning electron microscopy. The variation in drug loadings from 20 to 70 % w/w did not produce any perceptible change in the shape of indomethacin-loaded polystyrene microparticles (Figure 1A). However, the appearance of some roughness on the microparticle's surface was seen at 70 % w/w drug loading (Figure 1A) which could predict the drug crystal formation inside the microparticles. On the other hand, the shape of ibuprofen-loaded polystyrene microparticles was found to be influenced by the drug loadings (Figure 1B). When the drug loadings were less than 50 % w/w, the microparticles were spherical, above this drug loadings, the microparticles become irregular in shape. If a drug is completely soluble in the polymer solution, a pressure difference between the disperse phase and the continuous phase exists,

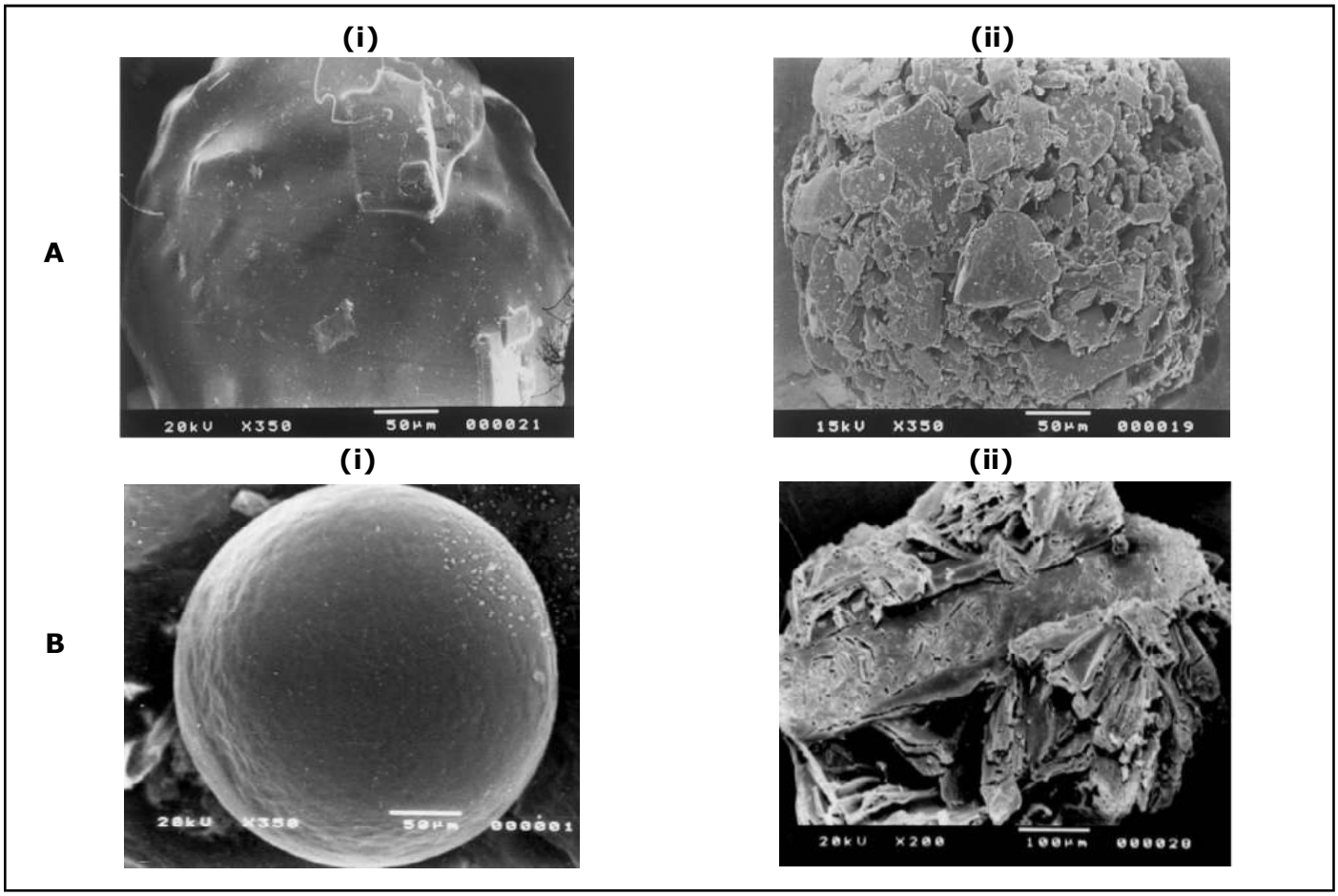

Figure 1. Scanning electron micrographs of polystyrene microparticles loaded with (A) indomethacin (i. 20 & ii.70 % w/w) and (B) ibuprofen (i. 20& ii.70 % w/w).

and the greater the pressure difference, the greater the distortion of the microparticles.[8] When ibuprofen loadings were less than 50 % w/w, the pressure difference between the two phases was not high enough to distort the spherical shape of the particles. Above these drug loadings, a considerable pressure difference between the two phases would have caused distortion of shape.

3.2. Case study-2: Development of an adjunct (formulation) to non-surgical periodontal therapy in the treatment of chronic periodontitis (Ornidazole-loaded polyethylene glycol-based microspheres)

Chronic periodontitis is an oral disease condition that occurs in teeth and their supporting structures.[9] Formation of pockets or pathologically deepened sulcus, leading to loss of teeth and destruction of tooth-supporting structures, caused by periodontal-pathogenic bacterial infections accompanied with sub-gingival plaque. Periodontal disease is also caused due to variation occurring in the microflora, histopathological variations, clinical symptoms and the location of the inflammation leading to deepened sulcus.[10] The early/moderate stage of periodontitis is termed "gingivitis" which is characterized by swelling, light bleeding and redness of marginal gingiva and in case chronic periodontitis and/or the final stage alveolar bone resorption occurs and supporting structures such as ligaments start detaching from teeth. About 80 % of the world's population is suffering from moderate to severe periodontitis. Treatments such as scaling and root planing are the options to prevent periodontal disease conditions.

Scaling is a process that comprises of elimination of calculus, plaque, tooth deposits and some stains. It can be done either above the gum (supra-gingival) or below the gum (sub-gingival). Root planing is a more radical treatment that involves the elimination of necrotic substances from the surface of the tooth under the gum, otherwise, it is similar to scaling. Undergoing scaling and root planing treatments only prevent the effects of periodontitis, it cannot clear the pathogenic bacteria colonizing in sub-gingival and supra-gingival spaces. Therefore, many drug delivery systems have been developed recently to cure periodontal diseases such as injectable drug delivery, bioadhesive gels, and adjunctive therapy which involves the application of various drugs-

Table 1. *In vitro* release profiles comparison for ornidazole (ORN) powder, ORN-loaded microspheres and ethylcellulose (EC)-coated ORN microspheres (Mean ± S.D).

Time (mins)	Cumulative percentage drug release from		
	ORN powder (mean ± SD, n=3)	ORN microspheres (mean ± SD, n=4)	EC-coated ORN microspheres (mean ± SD, n=3)
5	0.209 ± 0.365	0.155 ± 0.31	0.57 ± 0.03
15	59.94 ± 12.32	28.48 ± 11.75	7.31 ± 3.1
30	72.42 ± 18.18	56.24 ± 13.839	24.38 ± 5.5
45	100.64 ± 9.222	67.77 ± 7.328	41.45 ± 7.7
60	Not applicable	73.15 ± 5.04	42.25 ± 10.12

Table 2. Topical gels ingredients.

Ingredients	Formulation (% w/v) code					
	F*	F1	F2	F3	F4	F5
Ornidazole (ORN) drug powder	0.1	-	-	-	-	-
Ornidazole (ORN) microspheres or ethylcellulose (EC)-coated ORN microspheres	-	0.1	0.1	0.1	0.1	0.1
Carbopol 934	2	1	2	4	5	-
Hydroxy Ethyl Cellulose (HEC)	-	-	-	-	-	2.5
Distilled water	q.s	q.s.	q.s.	q.s.	q.s.	q.s

F* = control formulation containing only drug powder

incorporated microspheres, systemic antibiotics as well as antibiotics in combination with drugs.

Ornidazole (ORN) is a 5-nitro imidazole derivative with antiprotozoal and antibacterial activity against pathogenic anaerobic bacteria. Its antimicrobial activity is due to the reduction of the nitro group to a more reactive amine that attacks microbial DNA, inhibiting the further synthesis and causing degradation of existing DNA. Therefore, systemic and/or local application of ORN microspheres along with scaling and root planning treatments might be effective in the inhibition of colonial growth of patho-periodontic anaerobic bacteria so as to prevent and cure periodontal disease conditions. The ORN-loaded polyethylene glycol-based microspheres meant for topical delivery is more likely to provide the following two advantages (1) a better-controlled delivery of drug from ORN microspheres coated with or without ethylcellulose and after incorporation of drug-loaded microspheres into carbopol or hydroxyethyl cellulose-based gel formulation and (2) obtaining an adjunctive therapy thus preventing surgical procedure such as scaling and root planning over tooth cavity.

Adjunct/Adjunctive treatment is defined as another treatment that is carried out along with the primary treatment. Its purpose is to assist the primary treatment and is therefore generally called an "adjunct". Here, scaling and root planing are considered as a primary treatment option for chronic periodontitis whereas topical gels containing ORN-loaded hydrophilic polymer-based microspheres act as non-surgical periodontal adjunctive therapy. By combining both primary and adjunct therapies together, it is likely that the destruction of teeth-supporting ligament structure by pathogenic

microorganisms will be eradicated effectively. The microspheres were prepared based on two hydrophilic pharmaceutical excipients: polyethylene glycol and chitin. Interestingly, these two excipients have two different characteristics when they are used to develop injectable micro-and nano-formulations. Again, the preformed microspheres were coated with ethylcellulose (EC) polymer to make the ORN-loaded and EC-coated microspheres. To prepare the EC-coated microspheres, weighed amounts of ORN-loaded microspheres and EC were mixed in a ratio of 2:1. Using the TLC spraying apparatus, 20 ml of cyclo-hexane was sprayed onto the mixture containing the microspheres and EC. The resulting coated EC-microspheres were kept in the open air for drying over the time period of 12 hours.[11] Table 1 shows in vitro release profiles of ORN drug powder, ORN-loaded microspheres and EC-coated ORN-loaded microspheres. From Table 1, it is clear that coating of the microspheres with EC always showed the retardation of ORN release and incorporation of ORN into microspheres resulted in release retardation in comparison to ORN drug powder at all the studied dissolution time periods (5-60 minutes). Finally, the ORN powder and ORN-loaded microspheres with or without EC-coating were incorporated into two different gel bases as shown in Table 2.

Since the microspheres were prepared based on two hydrophilic polymers (PEG and chitin), it is more likely that a sufficient amount of ORN will be released initially from the microspheres into the gel base (due to the presence slightly hydrophilic structure of the selected gel base) and subsequently, the released drug will be available and even sufficient to act on the pathogens present over the applied buccal (gum) area instantly. This type of instant release of sufficient drug amount provides the prevention of the pathogens forming a buccal biofilm (association of single microbes into a colonized and non-penetrable form) that is more resistant to drug treatment and thus necessitate the treatment options such as scaling and root planing. If the hydrophobic polymers (instead of hydrophilic polymers) are used to prepare microspheres, then, the drug release is not to be instant (may be imminent) to reach the gel base. On the other hand, the question of using the drug incorporated in the gel base (rather than incorporating the drug-loaded hydrophilic polymer-based microspheres into the gel base) is again possible but we cannot expect the same/similar instant release of sufficient drug amount from the highly hydrophobic gel base only formulation. Here too, the question of selecting the hydrophilic or emulsion type gel base is possible and although a similar instant release of sufficient drug amount from the base can be achieved, the obtaining of a controlled drug release profile is not possible and the prepared hydrophilic gel-based formulation needs to be applied frequently into the buccal cavity. Usually, patients having periodontal disease condition are advised to use the drug-loaded gels at night time and the frequent application during the night time has thus associated with the problem of sleep deprivation. Hence on account of preventing the pathogenic microbes to become forming biofilm (or even calculus, plaque, tooth deposits and some stains) over the teeth area, a formulation strategy of providing initial instant release followed by controlled drug release could be a better option to act as an adjunct (formulation) to non-surgical periodontal therapy in the treatment of chronic periodontitis. And the results to show and fulfill the above-said initial instant release followed by controlled drug release via the currently proposed gel(s) containing the ORN-loaded hydrophilic polymer-based microspheres was tested via in vitro permeation study.

A previously reported method by Tamilvanan and Baskar (2013) to investigate the release of celecoxib from oil-in-water nanosized emulsion was followed to see in vitro release/permeation of ORN from drug-loaded gel and gel containing ORN microspheres or EC-coated ORN microspheres for 60 minutes using Franz diffusion cells.12 After incorporation into the carbopol or hydroxyethyl cellulose (HEC)-based gel, the ORN-loaded microspheres dissolved within 10 mins whereas EC-coated ORN microspheres showed very good stability in all the formulations up to over the time period of 30 to 45 minutes. As expected, all the gels containing ORN microspheres (formulation codes from F1 to F5) possessed the percentage drug permeation/release values in a phosphate buffer solution of pH 6.8 (Table 3) that were significantly lower when compared to the value (3.63 ± 0.34) obtained for gels containing ORN drug powder only (F*). Between the gels containing ORN microspheres and EC-coated ORN microspheres, the calculated value of percentage drug permeation/release was, however, found to be low for the formulation containing EC-coated ORN microspheres (Table 3). This indicates the EC coating onto the ORN microspheres had higher retardation of ORN release/permeation. A similar trend in the cumulative permeation percentage values was noticed for the gels containing ORN powder and ORN microspheres or EC-coated ORN microspheres when the permeation

Table 3. In vitro cumulative permeation percentage values obtained in a phosphate buffer solution of pH 6.8 for the gel formulations containing ornidazole (ORN) drug powder, ORN microspheres and ethylcellulose (EC)-coated ORN microspheres.

Formulation Code	Time (minutes)	Cumulative permeation percentage value obtained from gels containing		
		ORN drug powder (denoted as F*) (Mean ± S.D, n=3)	ORN microspheres (Mean ± S.D, n=3)	EC-coated ORN microspheres (Mean ± S.D, n=3)
F1	5	Not determined	1.688 ± 1.51	0.430 ± 0.58
	15	Not determined	5.676 ± 1.37	1.107 ± 0.78
	30	Not determined	12.62 ± 6.37	2.138 ± 0.51
	45	Not determined	18.277 ± 5.48	13.859 ± 1.50
	60	Not determined	24.120 ± 3.00	21.041 ± 2.10
F2	5	2.888 ± 2.51*	4.729 ± 2.80	0.256 ± 0.04
	15	7.676 ± 1.45*	7.131 ± 3.14	1.023 ± 0.15
	30	14.72 ± 5.37*	14.186 ± 3.40	5.493 ± 0.87
	45	22.677 ± 7.48*	16.724 ± 2.66	17.317 ± 1.63
	60	38.120 ± 3.00*	18.563 ± 2.11	18.618 ± 0.98
F3	5	Not determined	1.208 ± 0.44	0.267 ± 0.03
	15	Not determined	2.170 ± 0.55	1.942 ± 0.19
	30	Not determined	4.734 ± 2.37	4.035 ± 1.12
	45	Not determined	9.904 ± 1.01	7.843 ± 0.64
	60	Not determined	13.036 ± 1.07	9.867 ± 0.35
F4	5	Not determined	1.197 ± 0.26	1.206 ± 0.29
	15	Not determined	3.277 ± 0.93	2.181 ± 0.59
	30	Not determined	6.779 ± 2.77	4.729 ± 0.31
	45	Not determined	8.983 ± 2.76	5.514 ± 0.36
	60	Not determined	12.377 ± 1.88	8.811 ± 0.83
F5	5	Not determined	1.44 ± 0.19	0.741 ± 0.39
	15	Not determined	1.77 ± 0.56	1.585 ± 0.34
	30	Not determined	4.801 ± 1.21	2.865 ± 0.32
	45	Not determined	6.317 ± 2.30	4.197 ± 0.28
	60	Not determined	10.665 ± 3.21	5.444 ± 0.25

* Particular gels (denoted as F*) prepared from carbopol base (2.5% w/v) only and it contains only 100 mg ORN powder alone (one gram of gel containing 25 mg drug was taken for this study)

medium was changed from phosphate buffer solution of pH 6.8 to an artificial saliva solution of pH 6.8 (Table 4).

Case study-3: Influence of eutectic liquid on hydrophilic polymer-based micro systems

Multiple unit formulations consist of both monolithic (microspheres or microparticles) and reservoir (microcapsules) types. Whereas the monolithic type possesses an inseparable polymer and drug matrix, the presence of a distinct polymeric wall structure with a central drug core is inevitable for the reservoir type. With the help of protective colloids such as polyisobutylene (PIB), reservoir type particulate systems (microcapsules) are routinely being made.[13-15] But the PIB did not work when polyethylene glycol (PEG) was used to make reservoir

Table 4. In vitro cumulative permeation percentage values obtained in artificial saliva solution of pH 6.8 for the gel formulations containing ornidazole (ORN) drug powder, ORN microspheres and ethylcellulose (EC)-coated ORN microspheres.

Formulation Code	Time (minutes)	Cumulative permeation percentage value obtained from gels containing		
		ORN drug powder (denoted as F*) (Mean ± S.D, n=3)	ORN microspheres (Mean ± S.D, n=3)	EC-coated ORN microspheres (Mean ± S.D, n=3)
F1	5	Not determined	5.559 ± 0.32	1.814 ± 0.27
	15	Not determined	8.562 ± 0.63	5.392 ± 0.33
	30	Not determined	13.833 ± 0.65	9.696 ± 0.32
	45	Not determined	20.620 ± 0.66	19.614 ± 0.52
	60	Not determined	28.326 ± 0.64	24.229 ± 0.99
F2	5	4.87 ± 2.51*	0.967 ± 0.32	1.176 ± 0.34
	15	8.76 ± 1.45*	2.084 ± 0.43	1.869 ± 0.12
	30	15.64 ± 5.37*	3.047 ± 0.11	5.860 ± 0.51
	45	24.38 ± 7.48*	5.932 ± 0.29	7.978 ± 0.26
	60	38.12 ± 3.00*	6.100 ± 0.42	8.255 ± 0.98
F3	5	Not determined	2.082 ± 0.45	0.995 ± 0.25
	15	Not determined	3.895 ± 0.17	1.639 ± 0.54
	30	Not determined	8.242 ± 0.33	6.836 ± 0.59
	45	Not determined	13.691 ± 0.69	11.469 ± 0.57
	60	Not determined	16.469 ± 0.73	14.199 ± 0.42
F4	5	Not determined	2.082 ± 0.45	1.007 ± 0.06
	15	Not determined	2.923 ± 0.33	2.404 ± 0.50
	30	Not determined	7.941 ± 0.20	6.886 ± 0.33
	45	Not determined	10.195 ± 0.32	9.063 ± 0.21
	60	Not determined	11.326 ± 0.32	9.956 ± 0.19
F5	5	Not determined	1.567 ± 0.34	1.384 ± 0.16
	15	Not determined	3.725 ± 0.34	7.529 ± 0.28
	30	Not determined	6.166 ± 0.35	6.014 ± 0.17
	45	Not determined	8.79 ± 0.22	7.633 ± 0.35
	60	Not determined	12.5 ± 0.54	13.877 ± 0.29

* Particular gels (denoted as F*) prepared from carbopol base (2.5% w/v) only and it contains only 100 mg ORN powder alone (one gram of gel containing 25 mg drug was taken for this study)

type particulate systems and the produced particles appeared as monolithic type. With a view to find out an alternative to PIB in producing reservoir type particulate systems from PEG, Tamilvanan and Chanda (2019) have used a eutectic liquid consisting of a 1:1 ratio of camphor and menthol.[16] The peculiarity of developed micron-level particulate systems is the change in morphological behavior in the presence of surrounding aqueous medium (Figure 2). There are two different models described to indicate the change in morphological behavior of micron-level particulate systems on contact with an aqueous medium. While the first model describes the traditional way of dissolution medium permeation into the hydrophilic PEG matrix, the second model proposed is completely related to the participation/ presence of eutectic liquid in micron-level systems. Since both eutectic liquid and ORN are hydrophobic in nature, the ORN prefers to mix with eutectic liquid rather than in the PEG molecules. Indeed, it is reflected in the ORN entrapment efficiency value wherein the eutectic liquid plays important to achieve high entrapment of ORN (almost 15-20 % more) into the micro systems.[16]

Uncovered mechanistic insight of macroemulsions

It was around more than 50 years that colloidal drug delivery systems are being designed. Enhanced

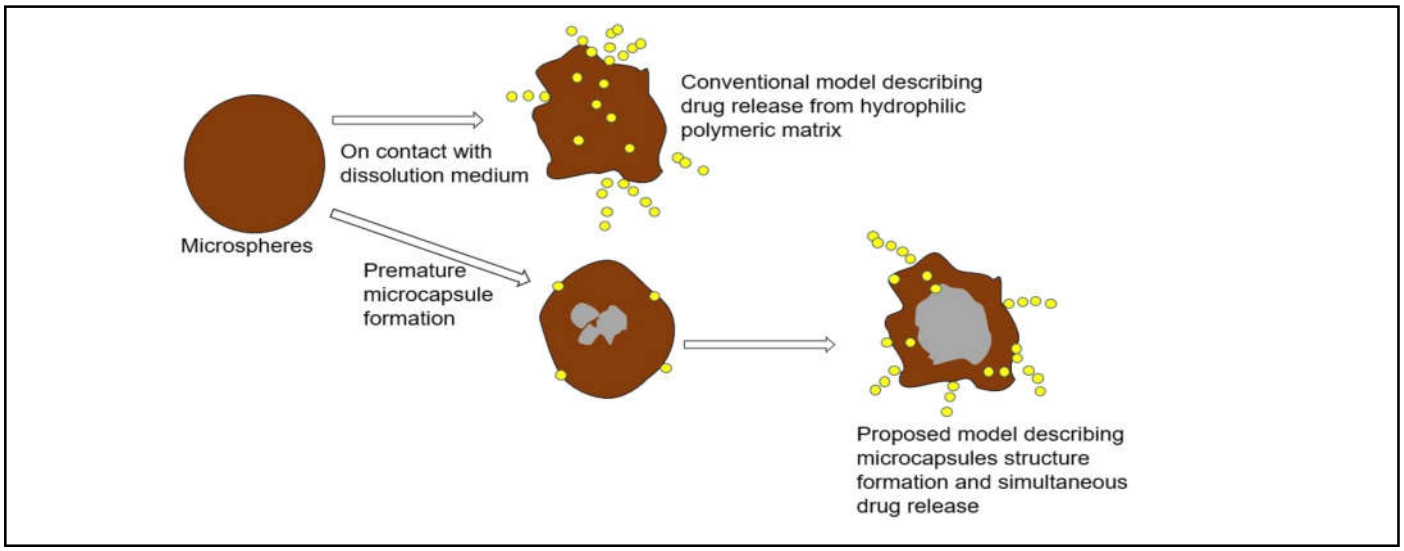

Figure 2. Proposed model describing the attitude of micron-level particles under dry and wet conditions

aqueous solubility value and elevated GI epithelial transport mechanism of hydrophobic drug molecules are easily achieved by using colloidal dispersions like cubosomes, ethosomes, liposomes, nanospheres, nanocapsules, niosomes, transferosomes, etc. In this list, the o/w emulsions possess the ability to hold both hydrophobic and hydrophilic drug molecules depending on solubility enhancement or GI epithelial transport elevation.[17,18] Between these two emulsions, the o/w emulsions have attractive multifunctional activities for medical and pharmaceutical applications. Even a sizeable number of commercial products are launched to correct various infectious diseases and reduce the myriad of symptoms related to the diseases.[19,20] Strictly speaking, the commercially available emulsions consist of dispersed oil droplets having a mean particle size ranging from 300 to 500 nm level. In general, the nano-sized particles produce a meta-stable form of particles due to the involvement of both multifunctional excipients and size-reduction machinery. The meta-stable nano-sized particles over the time period are slowly auto-converted into the stable particles having micron-level sizing i.e., macroemulsions.

Apart from incorporating the hydrophobic drug molecules into the emulsion dispersion system, it is also interesting to incorporate the water-soluble drug molecule into the dispersion system along with the hydrophobic drug moieties. Teixeira et al., in the year 2000 developed cationic emulsions consisting of the dispersed medium chain triglyceride (MCT) droplets stabilized with the three-different emulsifiers (Lipoid-E80, poloxamer-188 and stearylamine).[21] A peculiar 'handbag' architecture was found with the dispersed MCT droplets which is probably the first report of bi-compartmentalization within the emulsion system. By playing with an oil combination and a single emulsifier molecule, Leonardi et al., in the year 2015 created bicompartmental architecture on the dispersed oil droplets of o/w nanosized emulsions.[22] In the year 2019, Puri et al., developed the bicompartmentalized oil droplets by changing the ratio of two-different non-volatile oils and tried for the first time to entrap ginger powder, especially at one part of the compartment leaving another part for other hydrophilic drug.[23] Since then, the application of the dual drug-loading concept in the emulsions having bicompartmentalization on the dispersed oil droplets becomes a fascinating research work. Various terminologies have been ascribed to denote bicompartmentalization which include anisotropic, handbag, Janus and paired-bean.[24, 25]

Through the currently proposed o/w macroemulsions containing the bicompartmentalization on the dispersed oil droplets and prepared by low-shear size-reduction machinery, the following two advantages are being expected (1) the possibility to entrap two-different drug molecules possessing diverse physicochemical properties but similar therapeutic activity and (2) obtaining a synergistic therapeutic effect thus preventing the multiple time administration/application of the developed formulation for the treatment of disease like melanoma, for the management of inflammation produced due to Acne vulgaris and for the diminishment of pain severity during the primary dysmenorrheal condition.

Macroemulsion formulation development and characterization

With the help of laboratory scale mixing equipment

such as electric and magnetic stirrers, the o/w emulsions comprising bicompartmentalization on the dispersed oil droplets (micron-sized) can easily be made to meet the industrial aspirations of making a liquid-retentive topical macroemulsions for the treatment of skin melanoma, for the management of inflammation produced due to Acne vulgaris and for the diminishment of pain associated with the primary dysmenorrheal condition. Before making the macroemulsions, the selected drug molecules need to be processed either by a modified vapor pressure diffusion method or an anti-solvent precipitated method.[26, 27] The examples of drug molecules include adapalene (ADP), curcumin (CUM), fenugreek (FEN) or ginger (GIN). Since ADP possesses a highly fragile or unstable structure upon exposure to light and other processing conditions, unprocessed ADP drug particles are used throughout this study. The modified vapor diffusion method and anti-precipitation technique are utilized to prepare the processed samples for CUM whereas for the FEN and GIN, only the anti-solvent precipitation technique is applied. The selected non-volatile oils along with or without drug molecules may be mixed with a water phase containing single emulsifier molecules. Initially, the macroemulsions are prepared using synthetic surfactants (Tween 20 or Tween 80) and the formed emulsions contain the dispersed oil droplets with paired bean structures. The macroemulsions can also incorporate a phytomedicinal compound possessing the flavonoids/ polyphenolic structures like CUM, quercetin, rutin, GIN and FEN not only for their potential health benefits but also for their capability to position at the o/w interface and thus diminishing the interfacial tension between oil and water. The macroemulsions prepared with the amalgamation of the CUM crystals made by the modified vapor-diffusion method or with the incorporation of nanosized CUM particles prepared by the anti-solvent precipitation method are also able to produce a liquid-retentive emulsion containing the few paired bean-structured dispersed oil droplets (Figure 3A). The observed peculiar paired bean structure may also be further discriminated by incorporating another phytomedicinal compound (like asafetida or the lipophilic dye such as 6-coumarin having pale orange/very dark orange color) during the emulsion preparation step itself. So, the presence of paired bean structure might unequivocally be seen with two different colors provided by two different combinations of phytomedicinal compounds (CUM and asafetida, FEN and GIN or 6-cumarin). This invention thus indicates the distinguished properties of the paired bean structure having varied affinity to incorporate the medicinally-valued compounds derived from plant sources. Additionally, an anti-acne therapeutic agent such as ADP is also incorporated in its unprocessed form into the paired bean structure of the macroemulsions.

The typical formulas used to make macroemulsion consists of ADP, CUM, FEN and GIN are shown in Table 5. The mean particle diameters of the dispersed oil droplets of the macroemulsions were determined by using a Malvern master-sizer (Malvern, instrumentation limited; London, UK) by mixing 100-200 µl of emulsion with 150 ml of dispersion water (Hydro S) and the result is shown in Figure 3B.

The order of drug entrapment efficiency values observed in the macroemulsions was found to be CUM processed by anti-solvent precipitation technique (80.03 ± 0.505) > CUM processed by modified vapor pressure diffusion method (75.63 ± 1.14) > unprocessed CUM (46.83 ± 0.24) (Table 6). Similarly, the amount of drug present at the oil-water interface of the macroemulsion also showed a higher percentage value for processed CUM-entrapped emulsions than the unprocessed drug-loaded emulsion. The observed higher drug entrapment and/or drug accumulation at the oil-water interface of the macroemulsions could be attributed/corroborated with the presence of an amorphous, pure crystalline or size-reduced form of the drug in the macroemulsion systems.

Macroemulsion stability

The appearance or disappearance of paired bean structures inside the macroemulsion is monitored by keeping the macroemulsion at 37º C (room temperature) and 25º C (refrigerator) over the period of 4 weeks. Figure 4 depicts the optical microscopic pictures taken at different weeks after the storage of macroemulsions at two different temperature conditions. The macroemulsions are stable until the third week of storage time at both temperature conditions, that too, by keeping the paired bean structure intact into them.

Therapeutic potential of macroemulsion

In vitro anti-inflammatory activity of macroemulsion containing the FEN or GIN

By following the method of Chandra et al. (2012) and adopted previously by Tamilvanan and Kaur (2016), the in vitro anti-inflammatory activity of unprocessed and processed FEN or GIN powder and 1ml of olive-and silicone-oils-based macroemulsions

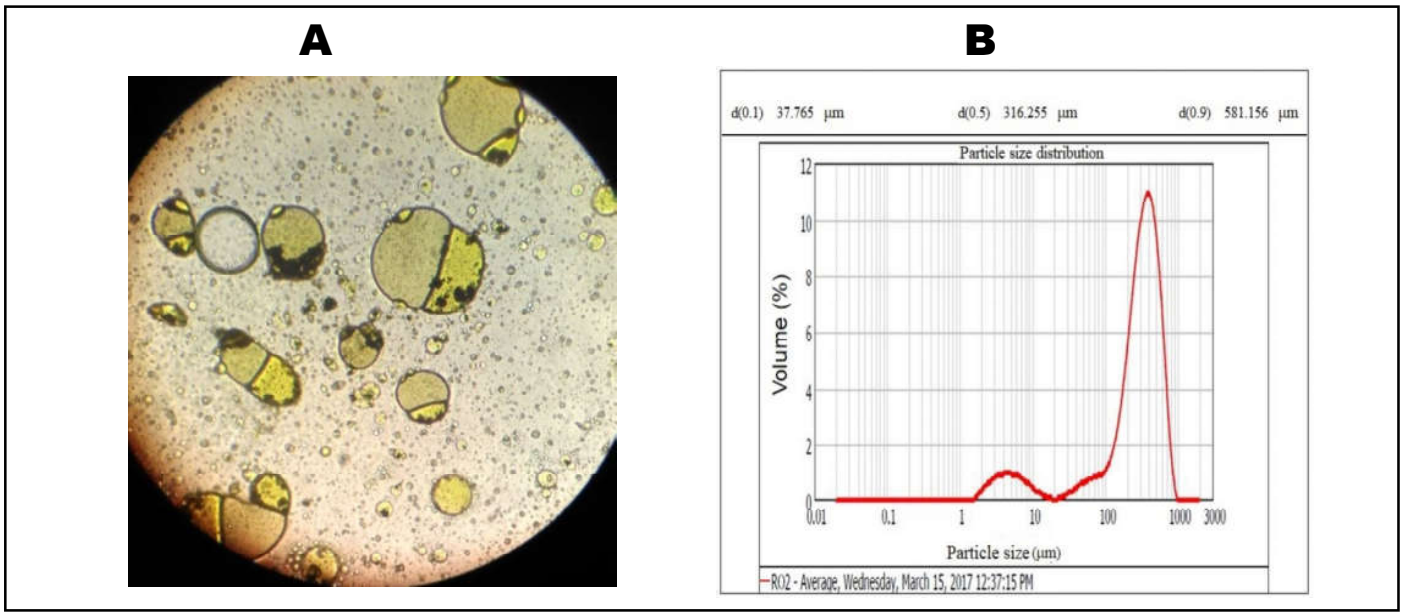

Figure 3. Optical microscopic pictures of curcumin-loaded macroemulsions containing the paired beans structure of dispersed oil droplets (A) and particle size analysis (d (0.5) = 316.255 µm) by Malvern Master-sizer (B).

Table 5. Typical formulas used to prepare macroemulsions containing unprocessed adapalene (ADP) and both processed and unprocessed curcumin (CUM), fenugreek (FEN) or ginger (GIN).

Drug name	Water phase		Oil phase	
	Water (g)	Tween 80 (g)	Olive oil (g)	Silicon oil (g)
Unprocessed ADP (40 mg)	9.782	0.408	1*	2
Processed and unprocessed CUM (40 mg)	19.56	0.40	1	4
Processed and unprocessed FEN (50-250 mg)	9.48	0.52	1	4
Processed and unprocessed GIN (50-250 mg)	9.48	0.52	1	4

* Castor oil (g), Processed means via modified vapor diffusion method or antisolvent precipitated method and unprocessed means natural structure of CUM, FEN or GIN

Table 6. Drug entrapment efficiency and drug amount at oil-in-water interface calculated for curcumin (CUM)-laden macroemulsions.

Compound CUM	Drug entrapment efficiency (%)	Drug amount at o/w interface (%)
Natural CUM	46.83	15.29
Modified vapour pressure diffusion method processed CUM	75.63	24.32
Anti-solvent precipitated method processed CUM	80.03	47.115

(equivalent to 9 mg of unprocessed or processed FEN or GIN powder) were determined.[28, 29] To avoid the use of laboratory animals for accessing the anti-inflammatory potential of drug-loaded macroemulsions, the protein denaturation assay can be used. Because the production of auto-antigens in certain inflammatory or arthritis diseases may be due to the denaturation of proteins in vivo.[30, 31] This test would be an indirect way of assessing the anti-inflammatory potentials of plant-derived active

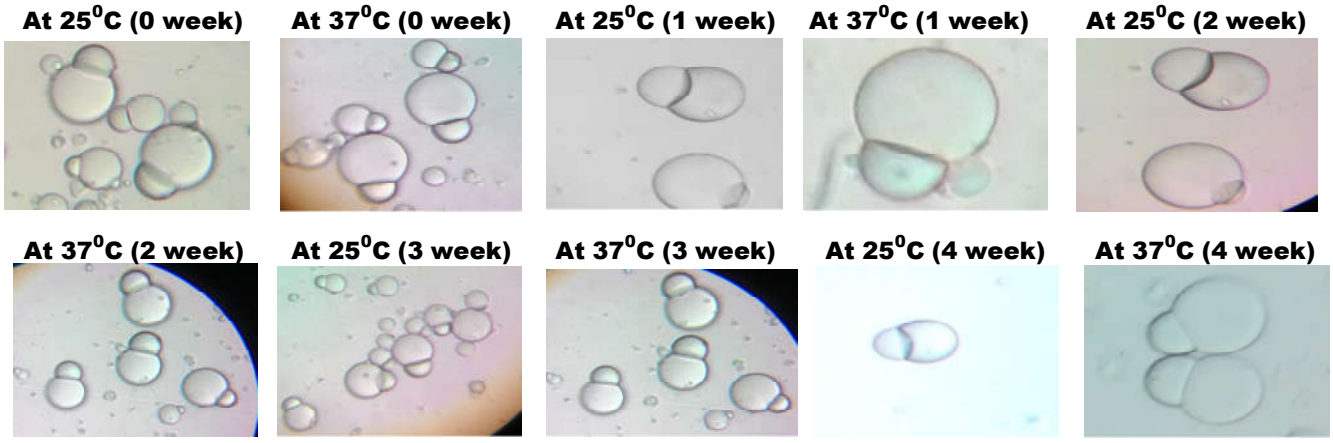

Figure 4 Optical microscopic pictures of macroemulsions stored at 25^0 and 37^0 C for 4 weeks.

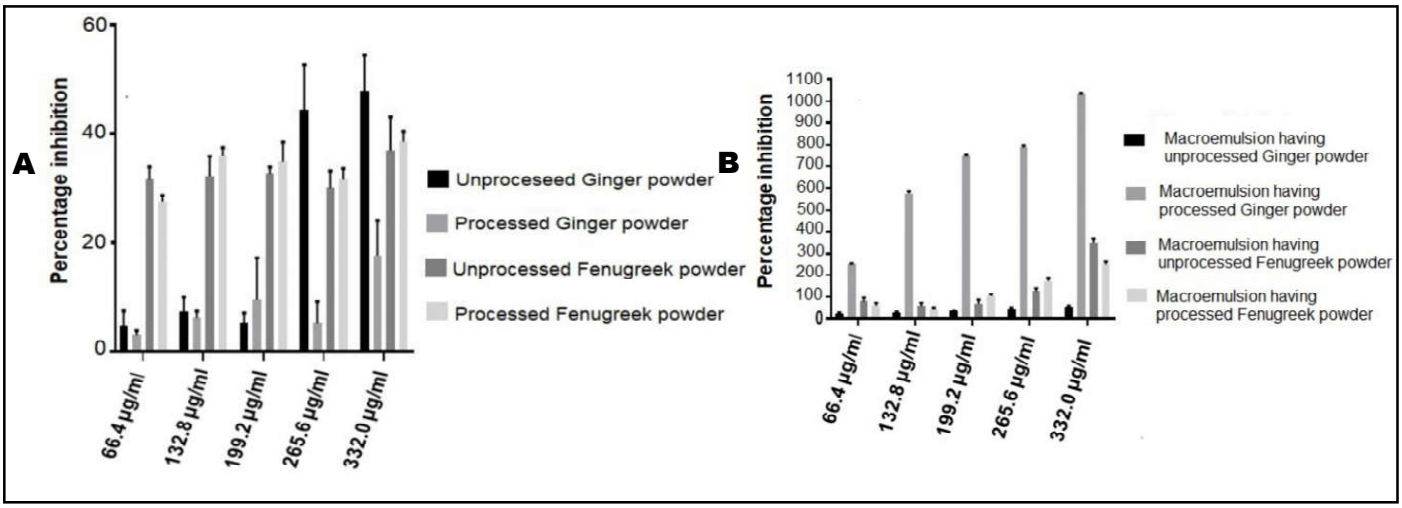

Figure 5. *In vitro* protein denaturation assay-based anti-inflammatory activities observed from (A) pH 7.4 phosphate buffer solutions containing unprocessed and processed fenugreek (FEN) or ginger (GIN) powder and (B) macroemulsions containing unprocessed and processed FEN or GIN powder at varying concentration levels (66.4, 132.8, 199.2, 265.6 and 332 µg/ml).

constituents, especially at their preliminary screening time.[28] The best formulation or good anti-inflammatory active constituents could be judged based on the higher percentage of protein inhibition at the lowest possible concentration level.

Figure 5 A&B depict the percentage inhibition values of protein denaturation reaction for Janus emulsions containing processed and unprocessed FEN or GIN (test formulations) and pH 7.4 phosphate buffer solutions containing processed and unprocessed FEN or GIN (control solutions). A concentration-dependent effect (from 66.4 to 332 µg/ml) on the percentage inhibition value of protein denaturation reactions at in vitro conditions was noticed. Furthermore, the percentage inhibition values for the protein denaturation reactions observed with test formulations were always higher than the values shown by controls. Therefore, macroemulsions containing the processed FEN or GIN could be of clinical interest for managing the inflammations produced at primary dysmenorrheal conditions.

Conclusion

The presence of API in two different physical forms/ states within the hydrophilic polymer-based microparticles influences the performances of API. The non-conventional application potential of hydrophilic polymer-based microparticles as an adjunct therapy is shown in this review. The compartmentalization made on the dispersed oil droplets of o/w macroemulsions put further be substantiated at the dispersed oil droplets in the nano-level category as well as incorporating the dual drug into the emulsion system to elicit a synergistic pharmacological activity for the management of other syndromes at reduced doses.

Future scope

The current review article explores the use and unique properties of hydrophobic eutectic liquid to

create microcapsule structures at in vitro conditions on contact with dissolution medium and thus the drug release retardation from the hydrophilic polymer matrix. Nevertheless, the microcapsule structure formation capability provided by the eutectic liquid needs to be tested at in vivo conditions after the insertion of hydrophilic polymer-based implants into the human body surfaces in the vicinity of body fluids. If it works, then, the in situ-forming microcapsules concept from hydrophilic polymers due to the presence of eutectic liquid needs further investigation. The current review also envisions the two-compartment structure in the dispersed oil droplets of macroemulsions. Such macroemulsions open the entrapment possibility of two different APIs possessing similar therapeutical activity but dissimilar physicochemical properties. Indeed, the exploration of two drugs-loaded macroemulsions for managing inflammatory bowel diseases, non-alcoholic fatty liver disease, etc., and inflammation-producing topical and ocular syndromes are currently undergoing in the research laboratories.

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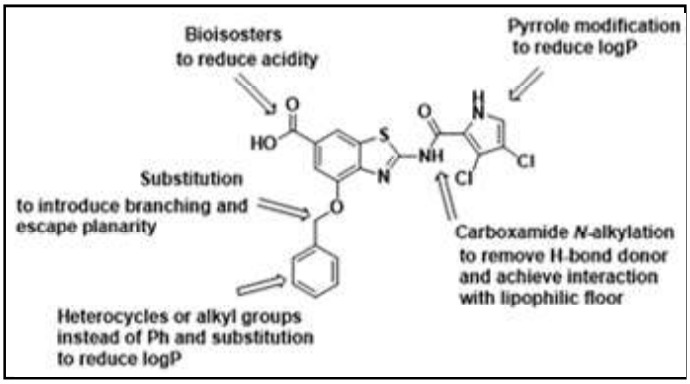
CRIPS Digest

Design and synthesis of Benzthiazole analogs as DNA gyrase inhibitors with potent activity against Pseudomonas aeruginosa and Acinetobacter baumannii

Bacterial topoisomerase IV (topo IV) and DNA gyrase are one of the most important targets for antibiotics as they are responsible for generation of negative



Prototype structure of Benzothiazole analogs targeting bacterial DNA gyrase

supercoils compelled by ATP and followed by Supercoil relaxation driven by ATP. Gyrase is composed of two subunits, GyrA and GyrB, while topo IV is composed of two subunits, ParC and ParE. There is currently no promising therapeutic application for antibiotics that target the ATP binding to the ParB/GyrB subunits. To achieve this, a novel class of DNA gyrase inhibitors based on benzothiazoles was designed, synthesized, and tested for antibacterial efficacy against six nosocomial pathogens that are members of the ESKAPE group.

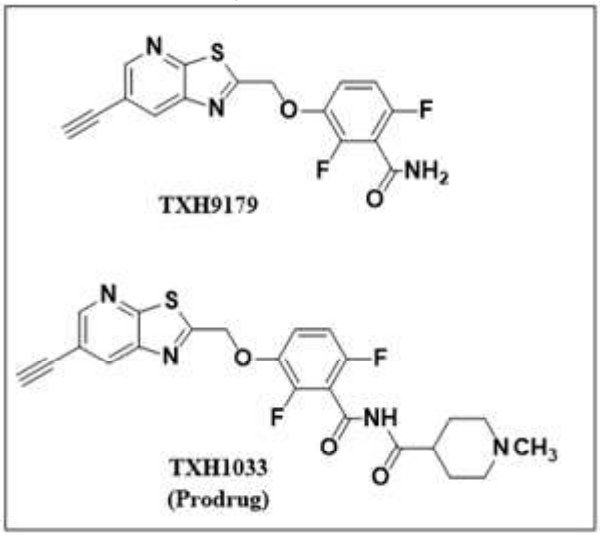
In order to increase the potency and pharmacokinetic properties of the benzthiazole analogs several modifications on the basic structure were carried out. In total 70 compounds were designed and synthesized and among them one of the compounds showed most potent antibacterial activity against the multidrug resistant class of bacteria.

In this lead compound, the benzylic proton was substituted with a methyl group. Due to this that benzylic carbon centre becomes chiral and it's found out that (S)-enantiomer of the lead compound showed more potent antibacterial activity than the other enantiomer. This lead compound showed remarkable inhibitory action against the DNA gyrase of P. aeruginosa (IC_{50} < 10 nm), *E. coli* (IC_{50} < 10 nm), and also showed excellent inhibitory action against topo IV of *P. aeruginosa* (IC_{50} = 29 nm) and *A. baumanni* (< 10 nm). To observe the binding of both enantiomers (S and R) to the ATP binding domain of the gyrase in *A. baumanni* and *P. aeruginosa*, a molecular docking study was carried out. The results of the docking experiment indicated that the (S)-enantiomer has a higher binding affinity for the various amino acids found in the Gyrase cavity than the (R)-enantiomer.

J. Med. Chem, 2023, 66, 1380-1425.

Structural and Antibacterial Characterization of a New Benzamide FtsZ Inhibitor with Superior Bactericidal Activity and *In Vivo* Efficacy Against Multidrug-Resistant Staphylococcus aureus

A strain of *S. aureus* resistant to methicillin (MRSA) stands as a clinically significant pathogenic bacterium. The resistance of clinical isolates of MRSA obtained from hospitals and communities to contemporary standard-of-care antibiotics such as linezolid and vancomycin is on the rise.



This article reported studies on TXH9179, a novel thiazolopyridine-benzamide FtsZ inhibitor of the next generation, which has a 6-acetylene substituent attached to the thiazolopyridine nucleus. The authors compared TXH9179's antistaphylococcal potency to that of two previously discovered thiazolopyridinebenzamide FtsZ inhibitors, each of which had a 6-Cl substituent (PC190723) or a 6-CF_3 substituent (TXA707).

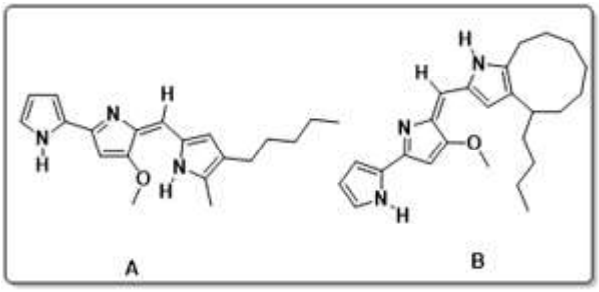
Against a library of 55 clinical strains of MRSA and methicillin-sensitive *S. aureus* (MSSA), including MRSA isolates resistant to vancomycin and linezolid, TXH9179 was found to be four times more potent than TXA707. TXH9179 was also associated with a reduced incidence of resistance compared to TXA707 in all except one of the MRSA and MSSA isolates investigated; the observed resistance was caused by mutations in the FtsZ gene. The alterations observed in MRSA's cytoskeleton, cell division process, and FtsZ localization, induced by TXH9179, align perfectly with its inhibitory effects on FtsZ.

Crystallographic investigations reveal the crucial molecular connections that facilitate complex formation while demonstrating the direct binding of TXH9179 with S. aureus FtsZ (SaFtsZ). Even at 10 times the concentration that exhibited antistaphylococcal action, TXH9179 did not exhibit any mammalian cytotoxicity. Serum acetylcholinesterases rapidly catalyze the hydrolysis of the carboxamide prodrug TXH1033 into TXH9179. Remarkably, the intravenous and oral administration of TXH1033 demonstrated improved in vivo effectiveness in addressing a mouse model of systemic MRSA infection (specifically, peritonitis) when contrasted with the carboxamide prodrug of TXA707, namely TXA709. When taken as a whole, this work identifies TXH9179 as a novel benzamide FtsZ inhibitor that shows promise and should be investigated further.

ACS Chem. Biol. 2023, 18, 629-642

Exploiting Differences in Heme Biosynthesis between Bacterial Species to screen for Novel Antimicrobials

Porphyrins are condensed heterocyclic molecules playing a crucial role of stabilizing and reducing the toxicity of free metal cations (Fe, Ni and Co) biologically by covalently binding with them. However, Heme, an iron containing porphyrin, is biologically abundant complex amongst other metal cations serving as a cofactor in many enzymes catalytic processes involving protein syntheses which makes it as a potential target for the antibacterial strategy.



Potential inhibitors A) BCHP B) prodigiosin

Recently, a bacterial classification was made based on the cell wall framework to understand the bacterial pathogenesis and they are of two types I) monoderm bacteria which is characterized by the presence of thick peptidoglycan only, II) diderm bacteria which is characterized by the presence of -peptidoglycan between cytoplasmic membrane and outer membrane. They follow two different pathways former involved with the coproporphyrin-dependent pathway (CPD) latter with protoporphyrin-dependent pathway (PPD) for Heme biosynthesis. CPD pathway follows last three steps: I) decarboxylation II) Oxidation III) insertion of Iron with different orders as that obtained in PPD pathway mediated by diderm bacteria. Monoderm bacterial pathogenesis can be controlled due to its distinct pathway, unlike that obtained in Host, of Iron-porphyrin complex formation. A set of library compounds was screened onto the recombineering *E. coli* strain *Sa*-CPD-YFP where diderm *E. coli* genes associated with PPD pathway were replaced with the genes involved in CPD pathway from monoderm staphylococcus aureus (SA) to realize which pathway is potentially inhibited. Nitrogen containing heterocycles were put into competitive bacterial viability assay and it was found out that tripyrrole class of drugs showed selective inhibition of CPD pathway of that hybrid *E. coli* strain due to structural planarity, electrostatic interaction and sufficient H-bond donor and acceptor properties. The observation became more conclusive, after control experiment performed with diderm E. coli strain (WT-CFP) and hybrid E. coli strain (sa-CPD-YFP), that it selectively inhibit CPD pathway to greater extent.

BCHP was able to reduce the bacterial growth by 4% of the sa-CPD-YFP strain and showed z factor of 0.76 which is considered as excellent value (Z > 0.5). The mechanism indicated significant dissimilarity in the order of the following three steps and the monoderm bacteria induced infection can be prevented with higher degree of selectivity and specificity by targeting and inhibiting monoderm mediated CPD pathway. In silico study was also carried out and it came with the agreement that

specific inhibition of ChdC enzyme occurred preventing the Heme formation.

Biomolecules **2023**, *13* (10), 1485.

Conformational Restriction: Requisite for Antibiotic drug discovery

Conformational restriction is well-precedented strategy involving the scaffold modification to develop new potential drug candidates. This rigidification minimise the entropy loss, improves the binding of drug to the target site, increases the selectivity of the lead compounds and also decreases the metabolic degradation. Quinolone antibiotics constitute a large group of broad-spectrum bacteriocidals that share a bicyclic core structure. Although offering excellent potency and wide spectrum action, the problem of resistance has encouraged the scientists to search for novel antibiotics, structurally related to quinolones. Increasing the restriction in the free movement of the bonds while moving from one generation to the other is one of the perspectives of having better activity and low resistance. Similiarly, in case of isoquinoline class of antibiotics, increasing the conformational restriction by replacing the double bonds with that of spiro ring improves the antibacterial activity against the strains of *Escherichia coli*, *Pseudomonas aeroginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *S. aureus*, *Enterococcus faecalis* and *Bacillus subtilis.* Spiro indoles forms the another class of compounds that are potent against *Klepsilae pneumonia*, *Bacillus cereus*, and *Salmonella typhi*.

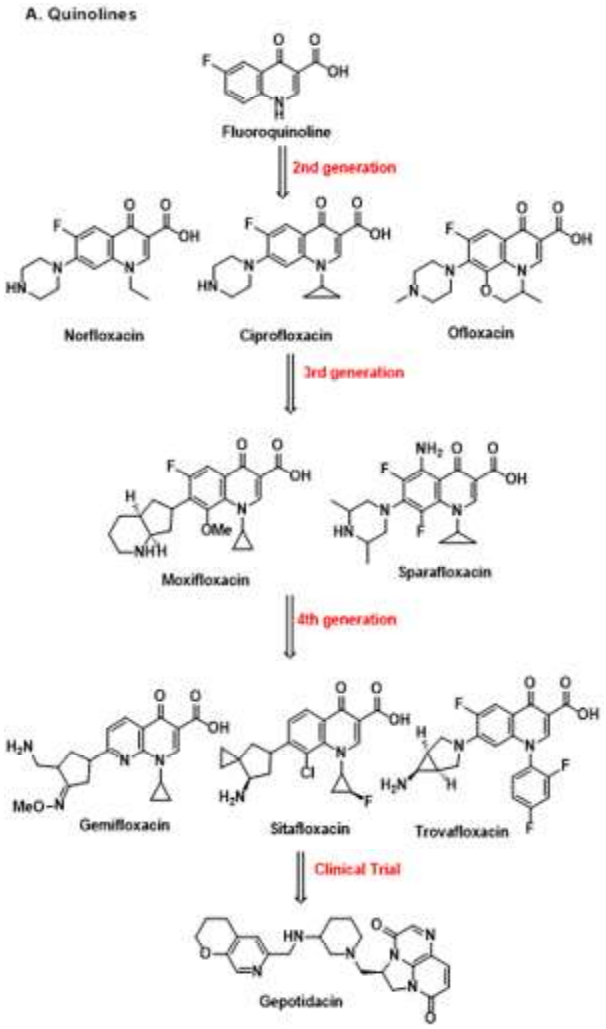


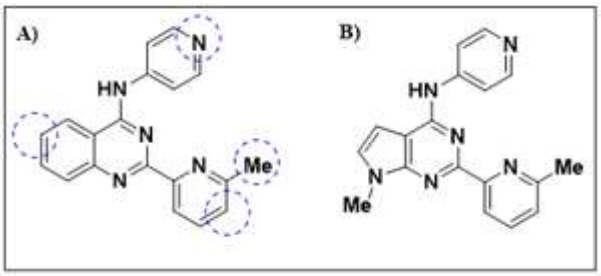
Fig. Conformational restriction leading to increased antibacterial activity.

These conformationally restricted molecular architectures help introducing specific molecular constraints in the lead candidate, allowing it to adopt the particular bioactive conformation and hence easy recognition and binding of ligand to the target.

Expert Opinion on Drug Discovery. **2020**, 3;15(5):603-25.
J. Antimicrob. Chemother., **2023**, 3;78(5):1137-42.

Dual Inhibitory activity of Anilinoquinazoline against *Mycobacterium tuberculosis* and the Host TGFBR1

The control on tuberculosis is slowed down due to increasing drug resistance in *Mycobacterium tuberculosis* (Mtb), thus strategies are required that specifically target drug resistance mechanism. A recent report showed that the responsiveness of T-cell is suppressed due to activation of TGFBR in the lungs. This report suggests that an approach to inhibit TGFBR in TB to restore CD4 T-cell function might be beneficial. Recently, a reported molecule, Anilinoquinazoline (AQA), found to be active against replicating, non-replicating, and drug-tolerant Mtb persisters along with inhibition of TGFBR1.



A) Metabolically susceptible sites of AQA. B) AQA analogue with metabolic stability

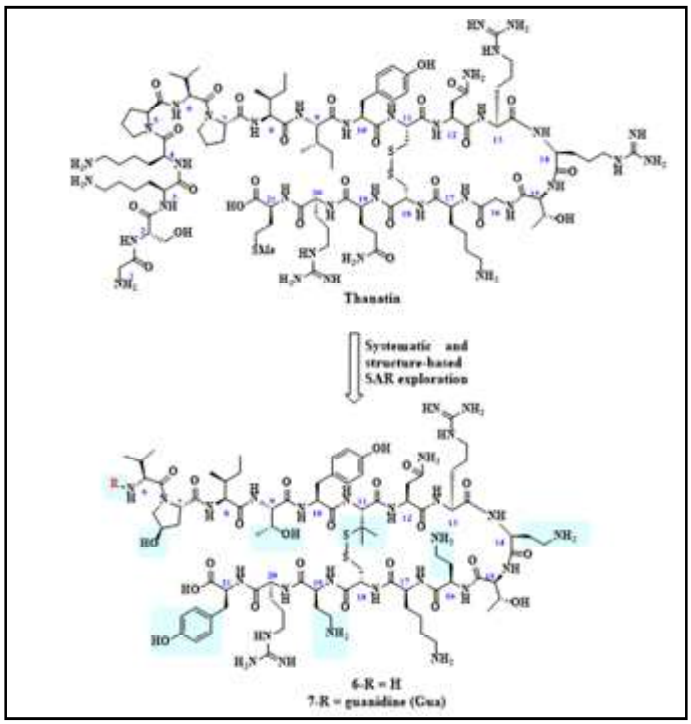
The SAR was established for this dual inhibitor and it was found that the pyridyl-6-methyl group is necessary for potent inhibition however it is also susceptible to metabolism by CYP450. By maintaining dual activity, pyrrolopyrimidine demonstrated balance of all three requirements. Additionally, metabolic stability and pharmacokinetic profiles were improved. Although it did not show the most potent inhibition, it demonstrated a balanced profile across these key

parameters. AQA is a highly intriguing compound with possibly many beneficial anti-Mtb properties in a single molecule. However, further testing is necessary to determine whether AQA has a dual activity against the host TGFBR1 and Mtb that is bactericidal to Mtb, including persisters.

J. med. Chem. **2023** DOI: doi.org/10.1021/acs.jmedchem.3c01273

Peptidomimetic Antibiotics Disrupt the Lipopolysaccharide Transport Bridge of Drug-Resistant Enterobacteriaceae

Antimicrobial resistance due to carbapenem-resistant Enterobacteriaceae (CRE) needs the attention of drug discovery scientists. Hence there is an urgent need to develop novel targets by replenishing antibiotics of standard-of-care (SoC). Lipopolysaccharide (LPS) transport (Lpt) machinery consists of seven Lpt A to G proteins in bacteria. This can serve as a target and can be interfered by naturally occuring peptide thanatin. It is a defense peptide consisting of 21–amino acid and exhibit broad-spectrum antimicrobial activity. Thanatin inhibit LPS transport across the periplasm having high affinity with proteins LptD and LptA. However due to shortcomings like rapid emergence of resistance and poor drug-like properties associated with it, we introduced thanatin-based synthetic macrocyclic peptides by tBu /Fmoc strategy using solid-phase peptide synthesis (SPPS). Disruption of the N-terminal helix in LptAm could be the reason for thanatin lessen binding to mutant LptAm^{Q62L}.



Structure of thanatin and modified antibiotic peptides 6 and 7 obtained through systamic and structure-activity relationship study. Structural modifications are shown in blue.

Screening contrary to a panel of LptA^{Q62L} clones combined with structure-based molecular design aided in recognizing lead compounds 5, 6, and 7 with enhanced antimicrobial potency. *In vitro* activity against Enterobacteriaceae revealed MIC90 values of 1, 0.5, and 8 mg/liter for compounds 6, 7, and thanatin respectively. Compound 7 was bactericidal against XDR and MDR Enterobacteriaceae including colistin and carbapenemase resistant strains with good drug-like pharmacokinetic and ADME properties, *in vitro* activity further translating into potent in vivo antimicrobial activity in infective mouse models. The approach of combining these attractive new antibiotics with standard of care antibiotics or alone could serve as treatment options to combat AMR.

Sci. Adv. **2023**, *9*(21),3683.

NSRS2023 – a report

NIPER-SAS Nagar organized a three days Symposium "NIPER Students Research Symposium-2023 (NSRS-2023)" from 10th to 12th August, 2023. This event was organized on the occassion of the silver jubilee of NIPER Act.

The National Institute of Pharmaceutical Education and Research (NIPER) SAS Nagar is governed by the Parliament act known as NIPER Act. This was enacted in 1998. This act changed many aspects of pharmaceutical sciences and education in India. The contribution of this pioneering decision by Govt. of India is responsible for the transformation of India into "Pharmacy of the world".

- Total number delegates (7 NIPERs): 220
- Research Scholar oral presentations : 26
- Research scholar poster presentation : 88
- Industrial expert presentations : 10
- Academic expert presentations : 28
- Alumini atteded : 25

Panel Discussion on The Role of NIPERs in shaping Pharmaceutical Sciences and Technology in India, deleberated by following panelists. The key suggestions that came out of this Session are:

1. NIPER system has done excellent groundwork in the field of pharmaceutical education -- but it is time for country to reap the benefits of the excellent training given by NIPERs.
2. The research at NIPERs should be able to touch the local problems and should provide possible answers for the local challenges being faced by our country. For example, in Punjab, Cancer is at troublesome levels. Hence, the NIPER, SAS Nagar should shift its focus to anti-cancer drug discovery. Similarly, the antibiotic drug resistance and avirus spread are recent troubles in India.
3. NIPERs should also form a strong relations with medical institutes as well as pharmaceutical industries which can foster the research and innovation in pharmaceutical areas.
4. Considering that the pharmaceutical sciences is addressing the societal issues, the Government agencies may be impressed to promote this applied field in the big way. DoP is rightly positioned to do this pioneering effort, as it has gained extensive experience and it is mentoring a network of NIPERs.
5. The Panel suggetsed for putting efforts on newer and upcomng raeserach area like QbD, AI/ML, bipharmaceticals/biosimilars etc.
6. NIPER should also put efforts in tranining skilled manpower to meet ever increasing demand of pharma industries.
7. During the discussion, it was recongnized that in the past four years, DoP became quite positively active and progressing in the direction. They expressed confidence, when these efforts progress in the direction, DoP may become a larger organization on par with DST and DBT; thus it can prositively impact the healthcare research in India.
8. India is already being considered as a pharmacy of the world, because it is catering to the medical needs of the third world countries. The sincere efforts of the Ministry of Chemicals and Fertilizers are going to provide further impetus and make India a true leader and a pioneer in Pharmacetucal Sciences.
9. NIPER should also improve its interactions with other meritorius organizations in India – the IITs, the autonomous institutions under DST, DBT, CSIR and ICMR.
10. NIPER-S should actively engage industry researchers in their teaching, such that the students get first hand information for the persons who solve the current problems.
11. NIPER-S should also improve international relations with the topmost pharmacy schools of the world.

On Day 2 there was a panel discussion on Current challenges in Pharmaceutical Industries and expectations from Academia.

- Dr Sudhir Sharma, Quantoom Biosciences, Belgium
- Dr C Subbarao, CRIUS life, Solan,
- Dr M Bommagani, Cadila, Ahemdabad,
- Dr Manoj Karwa, Aurigalife Sciences, New Delhi
- Dr Naveen jain, Panacea Biotech.
- Dr Srinivas Lanka, Chairman, APDC-NIPER Mohali

Virtual networking of chemical society to mitigate the chemical problem related to API synthesis, process safety, and process redesigning for cost-effectiveness and optimization, impurity troubleshooting

1. Better affordability of the government-funded Instrument at NIPERSs by reducing the price for measurements for the better affordability of MSME.
2. Establishment of NABL and GMP accelerated labs to help MSME.
3. Establishment of single point support kiosk for industrial cells to troubleshoot the problem of the pharma industry.
4. Showcasing the best research data on the institute's website to attract the industry.
5. Industrial houses spend time on currently active problems and academia focuses on fundamentals. To bring out synergy between these two, it is important to realize the strengths and weaknesses of both the parties.
6. A few MS / PhD research projects at NIPER may be based on current industrial needs in consultation with industrial scientists as co-supervisors.

Faculty members from NIPER Mohali presented their work on process and product development.

During the valedictory function of the NSRS2023 Prof. Panda appreciated the excellent presentations by the research scholars. Prof. Panda advised the researchers have real duty to return to society. The importance of AI tools in Pharmaceutical Sciences was emphasised by many speakers during NSRS2023. Lastly the awards were given to the winners.