Summary and Conclusion

Conventional multi-component non steroidal anti-inflammatory dosage forms constitute one of the largest groups of pharmaceuticals with a world market excess of $13 billion per annum due to increase the potency, multiple action and quick relief. Several multi-component non steroidal anti-inflammatory formulations are available in the market which is primarily used to treat pain and inflammation in musculoskeletal diseases, Alzimer's disease, rheumatoid arthritis, ankylosing spondylitis but the main problem with this conventional approach is that it produces lot of side effects like intestinal bleeding/ulceration, renal insufficiency, headache, dyspepsia, confusion, depression, broncho-spasm and rashes etc.

To overcome this conventional approach problems, the proposed work is to develop multi component microcapsular system by using various microencapsulation techniques and reformulate to in multiple unit system for a combination of non-steroidal analgesic and anti-inflammatory drugs. This system is able to maintain and control the plasma concentration without the need for frequent dosing and less side effects unlike in case of conventional dosage forms and increases the patient compliance.

The concept of multi-component therapy is beneficial when the selected agents posses differing mechanism of action that provide additive or
synergistic efficacy, reducing the required doses of individual agents as compared with monotherapy and potentially limiting side effects. Multicomponent therapy may seem costlier than monotherapies in the short term; but causes significant savings, lower treatment failure rate, lower case-fatality ratios, reduction in development of resistance and consequently less money needed for the development of new products in long-term therapy.

The rational fixed dose combination of nimesulide and tizanidine hydrochloride, aceclofenac and tizanidine hydrochloride are available in the market for the relief of inflammations and spasticity associated with multiple sclerosis, stroke, spinal cord injury, or disease as they fall in the similar range of pharmacokinetic profile. The half-lives of nimesulide (1.56 to 4.95 hr), aceclofenac (4-4.3 hr) and tizanidine (2.12 to 4.2 hr), fall in the same range and hence, the time course of action of the two drugs might be similar, which is an important criterion for the possibility of a rational fixed-dose-combination available in the market e.g., ITZGON-MR (Kee Pharma.), NIMETIZ (Ajanta Pharma.), NIMSAID-T(Medley), NIMULID-MR (Panacea Biotech.) and (Acent-TZ, Intra-Labs) etc. Various inflammatory painful conditions in which NSAIDs are used are often accompanied with muscle spasm. Since, Tizanidine is a myotonolytic agent and is helpful in management of muscular spasm, the combination of the two drugs will be helpful in managing such conditions. Tizanidine hydrochloride has been found to possess antinociceptive activity in animal models as well as clinical trials. This drug is until under investigation and limited literature are available. It is seen that the overall consumption of NSAIDs for the
management of pain is reduced when Tizanidine is given in combination to NSAIDs. Tizanidine hydrochloride also helps in improving the gastric safety profile of NSAIDs when given with them. Evidence is now emerging that tizanidine may also have a role to play in the treatment of other conditions associated with muscle spasm. Hence, it can be said that combination of tizanidine hydrochloride with aceclofenac or Nimesulide show synergistic potential which is one of the most important factors in deciding the feasibility. The main technological significance of this investigation was to develop microcapsular delivery systems and reformulate into multiple unit system to control and extend the release of the active ingredient from the microcapsules without attempting to modify the normal biofate of the active molecules in the body after administration and absorption. Furthermore, these dosage forms are less susceptible to dose dumping than the reservoir or matrix type, single unit tablet, since the drug release profile does not depend on the drug release properties of a single unit. Microparticles can be filled into hard gelatin capsules or compressed into tablets. This research helps to community regarding to avoid patient non-compliance problems and will provide delivery of drug in their usual forms with application of new techniques.

6.1 Microcapsular system:

It is a very simple, flexible, rapid and reproducible technique than other novel techniques which can be utilized to reformulate the current available formulations without any extra burden to Pharma companies. In the present
study we have choose very economic polymers for preparation of this system.

6.1.1 Aceclofenac

6.1.1.1 Cellulose acetate system

Microparticulate systems of aceclofenac (AC) were prepared by modified solvent evaporation method using different functional variables such as polymer (cellulose acetate, CA): drug ratios (1:9, 1:6, 1:3, and 1:1), agitation speeds (500-1500 rpm) and stirring time (5-15 min). The effects of processing variables were evaluated for physical parameters such as microparticle size, size uniformity, flowability, compressibility and entrapment efficiency. The average microparticle size increases from 80.2±1.45 to 97.3±2.06 µm with increase in the polymer concentration from 1:9 to 1:1 while reduces with increase in agitation speed from 500 to 1500 rpm and stirring time fro 5 to 15 min. At the higher speed, irregular shape of particles was obtained. The highest entrapment efficiency, size uniformity, angle of repose (23.6±0.3degree) and compressibility index (13.8±0.7%) parameters were considered to optimized the processing variables of microparticles, found that 1:6 (polymer: drug ratio), 1000rpm and 10min stirring time gives optimum physical properties of microparticles among all. To know the release pattern in the biological system; the in-vitro drug release study was carried out with all prepared microcapsules (AC-1 to AC-4) of various polymer concentrations, conventional tablets and compared the obtained data. The conventional tablet releases maximum drug within 3h while that
same amount of drug from microparticulate system releases in more than 12h. All formulations followed first order release kinetic and diffusion controlled drug release. No any drug polymer interaction was observed and all formulations were found to be stable under normal and accelerated stability conditions.

**Conclusion**

It concluded that the processing variables; polymer: drug ratio, agitation speed and stirring time affects the successful preparation of sustained release aceclofenac microparticles by an emulsion solvent evaporation process. These variables not only influenced the morphology but also affects on release of drug from the microparticles. The cellulose acetate is a suitable polymer to give diffusion-controlled microparticles. These microparticles also have flexibility to develop suitable dosage form for desired period of drug delivery as compared to available marketed formulation and improve safety, efficacy and patient compliance. These microparticles may reduce high dose absorption at once, which causes GI side effect as in conventional tablet.

**6.1.1.2 Ethylcellulose system**

Due to the easy availability and compliance with drug, ethylcellulose (EC) microparticulate systems of aceclofenac (AC) were also tried to improve pharmacological efficacy of the product. Microparticulate systems were prepared by solvent evaporation technique using different variables such as polymer (ethylcellulose): drug ratios (1:9, 1:6, 1:3, and 1:1 i.e., AC-1 to AC-4 respectively), agitation speeds (500-1000 rpm) and stirring time (10-30 min).
All microparticles evaluated for physical parameters as in case of cellulose acetate system. The average microparticle size increases from $72.59 \pm 1.72$ to $104.12 \pm 1.39$ µm with increase in the polymer concentration while reduces with increase in agitation speed and stirring time; and at the higher speed gives irregular shape of particles. The highest entrapment efficiency, size uniformity, angle of repose (22.4±0.5) and compressibility index (13.6±0.2) of microparticles were found with 1:6 (polymer: drug ratio), 800rpm and 20min stirring time among all microparticles. The in-vitro drug release study was carried out with prepared microparticles of various polymer concentrations and optimized processing variables and data were compared with conventional tablets. The conventional tablet releases maximum drug i.e., 94.33±1.5 % within 3h while microparticulate system releases 89.61±1.9% drug in more than 8h. FT-IR and other data showed no any drug polymer interaction and all formulations were found to be stable under normal and accelerated stability conditions ($4\pm1^\circ C$ (FT), $25\pm1^\circ C$ (RT) and $50\pm1^\circ C$ (HT)).

**Conclusion**

It concluded that the processing variables; polymer: drug ratio, agitation speed and stirring time affects the successful preparation of sustained release aceclofenac microparticles by an emulsion solvent evaporation process. These variables not only influenced the morphology but also affects on release of drug from the microparticles. The ethyl cellulose is a suitable polymer to give diffusion-controlled microparticles. These microparticles also
have flexibility to develop suitable dosage form for desired period of drug delivery as compared to available marketed formulation and improve safety, efficacy and patient compliance. These microparticles may reduce high dose absorption at once, which causes GI side effect as in conventional tablet.

6.1.1.3 Development of analytical methods

6.1.1.3.1 Spectrophotometry

In the present work, an UV spectrophotometric method has been developed for the estimation of aceclofenac in pharmaceutical dosage forms because no any analytical methodology is available in literature for physiological pH. The drug solution was prepared in alkaline phosphate buffer pH 7.4 and an absorption maxima was found at 273.5 nm. The Beer’s law was obeyed up to the concentration range of 5-45 µg/ml. The molar absorptivity was found to be 8.99x10³ l/mol/cm and Sandell’s sensitivity was found to be 0.0389 µg/cm²x0.001. The regression equation gave a slope of 0.0244 with correlation coefficient of 0.999. The precision and accuracy of the method was established by measuring six replicate samples of the drug in commercial formulations. None of the excipients of the formulation interfered in the analysis of aceclofenac by this proposed method. The results obtained by the proposed method were in good agreement with the labeled amounts. Performing recovery experiments using standard addition method checked the accuracy of the proposed method. The percentage recovery was close to 100%.
Conclusion

The proposed method is simple, convenient, accurate, sensitive and reproducible. Hence this can be employed for routine analysis of aceclofenac in formulations.

6.1.1.3.2 Reverse phase high performance liquid chromatography

A simple, economical, fast, and precise reverse phase high performance liquid chromatographic method has been developed for the determination of aceclofenac in dosage form. To study the accuracy, reproducibility, and precision of the proposed method, recovery experiments were carried out. Limit of detection (LOD), 0.0018µg/ml and limit of quantitation (LOQ), 0.0054µg/ml for aceclofenac indicating the sensitivity of the method, were also determined. The coefficient of variance was found to be 0.86% for aceclofenac that shows the method is highly precise. A fixed amount of the pre-analyzed sample was taken and standard drug was added at three different levels. Each level was repeated at least three times. The values of percent recovery (99.59-99.97%) indicate that the method is accurate, reproducible, and precise. The mobile phase, bearing methanol was found to be ideal. The elution was observed (RT-2.62 min). The values of percent recovery and standard deviation indicate that the method is accurate, reproducible, and precise.

6.1.2 Tizanidine hydrochloride
6.1.2.1 Cellulose acetate system

To formulate the oral drug delivery microencapsulated controlled release preparations of tizanidine hydrochloride (TIZ) were tried. Such designed systems are able to maintain plasma concentration without the need of frequent dosing and reduce side effects as in case of conventional dosage form. Microcapsules were prepared by modified solvent evaporation technique using different proportions of cellulose acetate. The microcapsules (TIZ1, TIZ2 and TIZ3) were compressed into tablets (T-TIZ1, T-TIZ2 and T-TIZ3) for oral delivery. The prepared microcapsules were evaluated for physical parameters and found white, free flowing and spherical in shape with the particle size varying from 175.92±9.82 to 194.94±14.28 µm. The in-vitro release study was carried out and t_{60}% of TIZ release from microcapsules was found to be 2.39±0.6, 3.39±0.6 and 4.55±0.8 h respectively for formulation TIZ1, TIZ2 and TIZ3 while their tablets i.e., T-TIZ1, T-TIZ2, T-TIZ3 and marketed SR were in 5.28±1.5, 6.86±0.6, 8.25 ±0.6 and 3.75±1.20 h respectively indicating more extension of time in tablet than microcapsules. The mechanism of drug release from tizanidine microcapsules and their tablets were studied by using Higuchi and Korsmeyer - Peppas models. The r-value for TIZ1, TIZ2 and TIZ3 indicates diffusion controlled with first order kinetic. The value of exponent coefficient (n) for T-TIZ1, T-TIZ2 and T-TIZ3 were found to be 0.844, 0.901 and 0.914 indicating anomalous, case-II and case-II transport release mechanism respectively. No any drug polymer interaction was observed and all
formulations were found to be stable under normal and accelerated stability conditions.

**Conclusion**

Tizanidine hydrochloride release matrices were prepared successfully utilizing cellulose acetate as a retardant material. From the technological point of view, microencapsulation followed by direct compression method enables the preparation of these matrices. The physical properties described for matrix tablets were found to be optimal for the manufacturing process. The release rate of tizanidine from microcapsules and their matrices were largely depends on the polymer concentration. As concentration of drug: polymer increases from 1:1 to 1:3, the release rate of TIZ was found to be decreased and release mechanism was shifted from anomalous to case-II transport with an increase in n-value. The study established that tablets made of CA microcapsules could be a good approach for controlled release zero order oral formulation of tizanidine.

**6.1.2.2 Ethylcellulose system**

Ethylcellulose (EC) microencapsulated preparations of tizanidine hydrochloride (TIZ) were prepared by modified solvent evaporation technique using different proportions of ethylcellulose as the retardant material to improve the efficacy of product. The microcapsules (TIZ1, TIZ2 and TIZ3) were compressed in to tablets (T-TIZ1, T-TIZ2 and T-TIZ3) for oral delivery. The prepared microcapsules were white, free flowing and spherical
in shape, with the particle size varying from 215.38±11.52 to 227.36±12.89 µm and shows good drug entrapment efficiency. The t_{60}% of TIZ release from microcapsules were found to be 2.15±0.6, 2.98±0.6 and 4.55±0.8 h respectively for formulation TIZ1, TIZ2 and TIZ3 while their tablets i.e., T-TIZ1, T-TIZ2 and T-TIZ3 releases in 7.49±1.2, 8.69±0.6 and 15.24 ±0.3 h respectively indicating more extension of time in tablet than microcapsules.

The mechanism of drug release from tizanidine hydrochloride microcapsules and their tablets were studied by using Higuchi and Korsmeyer - Peppas models. The r-value for TIZ1, TIZ2 and TIZ3 indicates diffusion controlled with first order kinetic. The value of exponent coefficient (n) for T-TIZ1, T-TIZ2 and T-TIZ3 were found to be 0.874, 0.902 and 0.913 indicating anomalous, case-II and case-II transport release mechanism respectively.

No any drug polymer interaction was observed and all formulations were found to be stable under normal and accelerated stability conditions.

**Conclusion**

Tizanidine hydrochloride release matrices were prepared successfully utilizing ethyl cellulose as a retardant material. From the technological point of view, microencapsulation and then direct compression method enables the preparation of these matrices. The physical properties described in table 4T-24 for matrix tablets were found to be optimal for the manufacturing process. The release rate of tizanidine hydrochloride from microcapsules and their matrices were largely depends on the polymer concentration. As concentration of drug: polymer increases from 1:1 to 1:3, the release rate of TIZ was found to be decreased and release mechanism was shifted from
anamolous to case-II transport with an increase in n- value. The study established that tablets made of EC microcapsules could be a good approach for controlled release zero order oral formulation of tizanidine hydrochloride.

6.1.2.3 Development of analytical methods

6.1.2.3.1 Spectrophotometry

Two simple, rapid, precise, highly specific and economical Spectrophotometric methods have been developed for the determination of tizanidine hydrochloride in its pharmaceutical dosage forms. Method A is based on the simple Spectrophotometric method with maximum absorbance at 319.5 nm in phosphate buffer 6.8 PH, obeyed linearity over 2-20 μg / ml. Method B is based on the difference Spectrophotometric method. The solutions of standard and pharmaceutical samples were prepared in 0.1 N HCl and 0.1 N NaOH. The absorbances were measured at 319.5 nm for acidic and 239.5 nm for alkaline solution. The difference between the absorbances of acidic and basic solutions was computed. The linearity range was found to be 2-12 μg / ml. Both the methods were validated and applied to the determination of tizanidine hydrochloride in solid dosage forms. It was concluded that the methods developed were accurate, sensitive, economical and precise for the quality control of tizanidine hydrochloride in pharmaceutical preparations.

6.1.2.3.2 Reverse phase high performance liquid chromatography

A simple, economical, fast, and precise reverse phase high performance liquid chromatographic method has been developed for the determination of
tizanidine hydrochloride in tablet dosage form. A C18 LUNA (5 micron 25 cm×4.6 mm) column from Phenomenex was in isocratic mode with mobile phase methanol. To study the accuracy, reproducibility, and precision of the proposed method, recovery experiments were carried out, limit of detection (LOD), 0.002µg/ml and limit of quantitation (LOQ), 0.006µg/ml for tizanidine indicating the sensitivity of the method were also determined. The coefficient of variance was found to be 0.62% for tizanidine that shows the method is highly precise. A fixed amount of the preanalysed sample was taken and standard drug was added at three different levels. Each level was repeated at least three times. The values of percent recovery were found to be 99.0-99.20 indicates that the method is accurate, reproducible, and precise. Experiments were carried out to establish the method. The mobile phase, bearing methanol was found to be ideal. The elution was observed (RT-4.51 min).

6.1.3 Nimesulide

6.1.3.1 Cellulose acetate system

Microparticulate systems of nimesulide (NIM) were prepared by modified solvent evaporation method using different processing variables such as polymer (cellulose acetate, CA): drug (NIM) ratios (1:9, 1:6 and 1:3), agitation speeds (500-1500 rpm) and stirring time (15-30 min). The effects of processing variables were evaluated by microparticle size and entrapment efficiency. The average microparticle size increases from 66.8±1.45 to 87.3±1.06 µm with increase in the polymer concentration while reduces with increase in agitation speed and stirring time; but at the too higher speed
gives irregular shape of particles. The highest entrapment efficiency (77.83±0.51%), size uniformity, free flowability, i.e., angle of repose (23.5±0.4°) and compressibility index (14.2±0.6 %), of microparticles were found with 1:6 (polymer: drug ratio), at 1000 rpm and 20 min stirring time among all prepared microparticles. All physical datas were statistically evaluated and found P ≤ 0.05. The in-vitro drug release study of microparticles with optimized processing variables (agitation speed and time) were carried out and compared with conventional and marketed SR tablets. The conventional tablet releases maximum drug within 4 h while microparticulate system releases same drug amount in more than 14h. All formulations followed first order release kinetic and diffusion controlled drug release (Higuchi model). No any drug polymer interaction was observed and all formulations were found to be stable under normal and accelerated stability conditions.

**Conclusion**

The study concluded that the processing variables; polymer: drug ratio, agitation speed and stirring time affects the preparation of sustained release nimesulide microparticles by an emulsion solvent evaporation process. These variables not only influenced the morphology but also affects on release of drug from the microparticles. The cellulose acetate could be a suitable polymer to give diffusion-controlled sustained formulation of nimesulide. Such sustained microparticles can also provide the flexibility to develop suitable dosage form for desired period of drug delivery as compared to available SR marketed formulation. Thus, it may improve
safety, efficacy and patient compliance with reduced common cause of problem due to high dose absorption at once in conventional tablet.

6.1.3.2 Ethylcellulose system

Microparticulate systems of nimesulide (NIM) were prepared by modified solvent evaporation method using different processing variables such as polymer (ethylcellulose, EC): drug (NIM) ratios (EC: nimesulide) 1:9, 1:6 and 1:3, agitation speeds (500-1000 rpm) and stirring time (5-15 min). The effects of processing variables were evaluated by microparticles size and entrapment efficiency. The average microparticles size increases from 65.53±1.02 to 97.3±2.06 µm with increase in the polymer concentration while reduces with increase in agitation speed and stirring time; but at the too higher speed gives irregular shape of particles. The highest entrapment efficiency (75.17±0.44 %), size uniformity, free flowability, i.e., angle of repose (27.5±0.3°) and compressibility index (16.1±1.1%), of microparticles were found with 1:6 (polymer: drug ratio), at 800 rpm and 10 min stirring time among all prepared microparticles (P ≤ 0.05). The in-vitro drug release study of microparticles with optimized processing variables (agitation speed and time) were carried out and compared with conventional and marketed SR tablets. The conventional and SR tablet releases maximum drug within 4 and 8h while microparticulate system releases in more than 14h. All formulations followed first order release kinetic and diffusion controlled drug release (Higuchi model). These microparticles are stable at room temperature.
(25±1°C) but agglomerate at elevated temperature (50±1°C) by softening and fusion of the polymer observed under SEM study. No any drug polymer interaction was observed and all formulations were found to be stable under normal and accelerated stability conditions.

**Conclusion**

The study concluded that the processing variables; polymer: drug ratio, agitation speed and stirring time affects the preparation of sustained release nimesulide microparticles by an emulsion solvent evaporation process. These variables not only influenced the morphology but also affects on release of drug from the microparticles. The ethyl cellulose could be a suitable polymer to give diffusion-controlled sustained formulation of nimesulide. Such sustained microparticles can also provide the flexibility to develop suitable dosage form for desired period of drug delivery as compared to available SR marketed formulation. Thus, it may improve safety, efficacy and patient compliance with reduced common cause of problem due to high dose absorption at once in conventional tablet.

**6.1.3.3 Development of analytical methods**

**6.1.3.3.1 Spectrophotometry**

In the present work, an UV spectrophotometric method has been developed in pH 6.8 phosphate buffer for the estimation of nimesulide in pharmaceutical dosage forms. Optimum operating conditions used in the procedures were established adopting one variable at a time. The absorption maximum was found to be 397 nm. The Beer’s law was obeyed in the concentration range of 5-40 µg/ml. The regression equation gave a slope of 0.347 and intercept
at 0.0353. The correlation coefficient was found to be 0.999. The precision and accuracy of the method was established by measuring six replicate samples of the drug in commercial formulations. None of the excipients of the formulation interfered in the analysis of nimesulide by this proposed method. The results obtained by the proposed method were in good agreement with the labeled amounts.

6.2 Development of multi-component microcapsular system of aceclofenac and tizanidine hydrochloride

6.2.1 Cellulose acetate based system

Bi-layered tablets of aceclofenac (AC) and tizanidine hydrochloride (TIZ) were prepared by using different proportions of cellulose acetate (CA) as the retardant material. Twelve formulations of bi-layered tablets i.e., F1 to F12 having different proportion of microparticles developed by varied proportions of polymer: drug ratio i.e., 1:9 -1:1 for AC and 1:1 – 3:1 for TIZ. The tablets contain microparticles equivalent to 150 mg of AC as maintenance dose and equivalent to 6 mg of TIZ will serve as loading as well as maintenance dose. The equivalent quantity of AC and TIZ were calculated on the basis of entrapment efficiency of AC and TIZ microparticles. Sustained release non-disintegrating tablets of AC and TIZ microparticles were prepared by direct compression of different concentration of microparticles of AC and TIZ separately. Then these non-disintegrating tablets were further double compressed with initial loading dose containing solid dispersion of AC granules (equivalent to 50 mg of AC) with polyethylene glycol-6000 (AC: carrier ratio, 4:1) prepared by conventional solvent evaporation method. The
Tableting properties flowability, compressibility, size and %drug content of all the granules were studied prior to compression. Each tablet contained equivalent to 200 mg of AC and 6 mg of TIZ. Physical characteristics and in-vitro release pattern of microparticles and its tablets were studied by using USP dissolution apparatus (USP XXVI/NF21, 2003) type 2 (paddle method) in simulated gastric fluid pH 2.0 and alkaline phosphate buffer pH 7.4. The drug content in release study was calculated by using developed spectrophotometric and reverse phase high performance liquid chromatographic simultaneous determination method of AC and TIZ. Various kinetic models viz., Higuchi and Korsmeyer-Peppas and first order were applied to know drug release pattern data, and found that both drugs microparticles and its tablet formulation (F1-F12) follows diffusion limited drug release pattern.

The in-vitro release data showed that more than 80.0 % of AC and 90.0 % of TIZ were released within 10.0-16.0 h for AC and 10.0-16.0 h for TIZ, which may be extended due to the microparticulate matrix tablets formulation. To observe the kinetic of drug release, the logarithm of percent drug retained in formulations verses time was plotted and found straight line (r2 values 0.9324-0.9916 and 0.9689-0.9921 for AC and TIZ respectively). It indicates that all formulations follows first order release rate kinetic pattern.

The first order rate constant K1 of formulation F1 to F12 were found to be in the range of K1=0.087-0.242 and 0.143-0.287 %hr-1for AC and TIZ respectively. The dissolution data of all formulations were fitted in Higuchi’s model and found, that was linear (r2 values 0.9317-0.9903 and 0.9445-
0.9711 for AC and TIZ respectively) indicated diffusion controlled release. The dissolution data of AC and TIZ tablets were also fitted to the well-known exponential equation (Korsmeyer -Peppas equation), which is often used to describe the drug release behavior from polymeric systems. All tablet formulations (F1-F12), the values of $n$ and the Log k for AC and TIZ ranged from 0.4603 - 0.5560 and 0.702 – 1.0112 and 1.2629 – 1.5116 and 0.8758 – 1.2931 respectively, indicating a release mechanism anomalous transport for the AC release and anomalous transport to non anomalous transport mechanism in case of TIZ. All formulations were found to be stable under normal and accelerated stability conditions.

Conclusion
The fixed dose combination of aceclofenac (AC) and tizanidine hydrochloride (TIZ) could be prepared by using multi-component microparticulate system for the relief of inflammations and spasticity. The multi-component tablets containing AC (1:6) and TIZ (3:1) "i.e.," formulation F9 showed almost identical release kinetics and it is expected that the time course of action of the two drugs might be similar in biological system, which is an important criterion for the selection of a rational fixed-dose-combination. Thus designing of multi-component sustained delivery of AC with TIZ in proper dosing services will minimize the drug toxicity, reduce the overdosing or drug complications, keep health care at minimum cost and ultimately increase the patient compliance.

6.2.2 Ethylcellulose based system
Bi-layered tablets of aceclofenac (AC) and tizanidine hydrochloride (TIZ) were also prepared with different proportions of ethylcellulose (EC) as the retardant material. Twelve formulations of bi-layered tablets i.e., F1 to F12 having different proportion of microparticles developed by varied proportions of polymer: drug ratio i.e., 1:9 -1:1 for AC and 1:1 – 3:1 for TIZ. The tablets contain microparticles equivalent to 150 mg of AC as maintenance dose and equivalent to 6 mg of TIZ will serve as loading as well as maintenance dose. The equivalent quantity of AC and TIZ were calculated on the basis of entrapment efficiency of AC and TIZ microparticles. Sustained release non-disintegrating tablets of AC and TIZ microparticles were prepared by direct compression of different concentration of microparticles of AC and TIZ separately. Then these non-disintegrating tablets were further double compressed with loading dose containing solid dispersion of AC granules (equivalent to 50 mg of AC) with polyethylene glycol-6000 (AC: carrier ratio, 4:1) prepared by conventional solvent evaporation method. The tableting properties of all the granules were studied prior to compression. Each tablet contained equivalent to 200 mg of AC and 6mg of TIZ. Physical characteristics and in-vitro release pattern of microparticles and its tablets were studied by using USP dissolution apparatus (USP XXVI/NF21, 2003) type 2 (paddle method) in simulated gastric fluid and alkaline phosphate buffer pH 7.4. The drug content and drug release were found to be calculated by using developed spectrophotometric and reverse phase high performance liquid chromatographic simultaneous determination method of AC and TIZ. Various kinetic models viz., Higuchi and Korsmeyer-Peppas
and first order were applied to know drug release pattern data, then found that both drugs microparticles and its tablet formulation (F1-F12) follows diffusion limited drug release pattern. The in-vitro release data showed that more than 90.0 % of AC and 90.0 % of TIZ were released within 08.0-14.0 h for AC and 14.0-24.0 h for TIZ, which may be extended due to the microparticulate matrix tablets formulation. To observe the kinetic of drug release, the logarithm of percent drug retained in formulations verses time was plotted and found straight line ($r^2$ values 0.9129-0.9819 and 0.9071-0.9575 for AC and TIZ respectively), indicates that all formulations follows first order release rate kinetic pattern. The first order rate constant $K_1$ of formulation F1 to F12 were found to be in the range of $K_1=0.186-0.374$ and $0.087-0.202$ %hr$^{-1}$for AC and TIZ respectively. The dissolution data of all formulations were fitted in Higuchi’s model and found, that was linear ($r^2$ values 0.9353- 0.9742 and 0.9448-0.9828 for AC and TIZ respectively) indicated diffusion controlled release. The dissolution data of AC and TIZ tablets were also fitted to the well-known exponential equation (Korsmeyer -Peppas equation), which is often used to describe the drug release behavior from polymeric systems. All tablet formulations (F1-F12), the values of $n$ and the Log $k$ for AC and TIZ ranged from 0.9589- 1.1119 and 0.7141–1.0285 and 0.8754–1.0119 and 0.6275–0.9482 respectively, indicating a release mechanism case-II transport for the AC release and anomalous transport to non anomalous transport in case of TIZ.
All formulations were found to be stable under normal and accelerated stability conditions.

Conclusion
The fixed dose combination of aceclofenac (AC) and tizanidine hydrochloride (TIZ) could be prepared by using multi-component microparticulate system for the relief of inflammations and spasticity. The multi-component tablets containing AC (1:1) and TIZ (1:1) "i.e.," formulation F1 showed almost identical release kinetics and it is expected that the time course of action of the two drugs might be similar in biological system, which is an important criterion for the selection of a rational fixed-dose-combination. Thus designing of multi-component sustained delivery of AC with TIZ in proper dosing services will minimize the drug toxicity, reduce the overdosing or drug complications, keep health care at minimum cost and ultimately increase the patient compliance.

6.2.3 Development of analytical methods

6.2.3.1 Spectrophotometry
A aceclofenac and Tizanidine hydrochloride is the leading prescribing combination (Acent-TZ, Intra-Labs) by the physician. Literature revealed that there is no combination formulation is present in the market, which provoke the preparation of dual drug delivery system containing aceclofenac and tizanidine in a single dosage form and develop the estimation procedure. So a simple rapid, accurate, economical and sensitive spectrophotometric
method for the determination of aceclofenac and tizanidine in combined
dosage forms has been developed. The method is based on the additivity of
absorbances. Aceclofenac shows absorption maxima at 273.5 nm and
tizanidine shows absorption maxima at 319.5 nm in phosphate buffer pH 7.4.
Beer Lambert’s law is obeyed for both the drugs in the concentration range
5-45 µg/ml. The accuracy and reproducibility of the proposed method was
statistically validated by recovery studies.

6.2.3.2 Reverse phase high performance liquid chromatography
The reversed-phase high-performance liquid chromatographic (RP-HPLC)
method has been developed to quantify aceclofenac and tizanidine
hydrochloride simultaneously in raw material and pharmaceutical
formulations. A C18 LUNA (5 micron 25 cm×4.6 mm) column from
Phenomenex is used in isocratic mode with mobile phase methanol. The
flow rate is 0.8 ml/min, and effluent was monitored by ultraviolet absorbance
at 294 nm with no interference of commonly used excipients. The retention
times of aceclofenac and tizanidine hydrochloride are 2.91 min and 4.62 min,
respectively. Linearity range for aceclofenac and tizanidine were 2.0-20
µg/ml (r=0.9974) and 0.4-4.0 µg/ml (r=0.9994), respectively. The linear
regression equations are \( Y = 14.779X + 5.5915 \) for aceclofenac, and
\( Y = 14.631X - 0.7926 \) for tizanidine hydrochloride. The high percentage of
recovery of the drugs, indicate that the method is highly accurate. The
content of the percentage of drugs in market samples indicate that the
proposed method is simple, rapid, precise, and accurate, for the estimation
of aceclofenac and tizanidine, in its pharmaceutical formulation. Precision,
reproducibility, and specificity, limit of detection (LOD), 0.0018µg/ml and 0.002µg/ml and limit of quantitation (LOQ), 0.0054µg/ml and 0.006µg/ml for aceclofenac and tizanidine hydrochloride respectively indicating the sensitivity of the method, were also determined. The coefficient of variance was found to be 0.62% for tizanidine and 0.86% for aceclofenac that shows the method is highly precise. The accuracy of the method was evaluated by carrying out recovery studies. For that, known concentration of standard solutions was added to the pre-analyzed sample solution, and the recovery was calculated and it is found to be 99.82-100.04% for AC and 99.87-100.12 for TIZ.

6.3 Development of multi-component microcapsular system of nimesulide and tizanidine hydrochloride

6.3.1 Cellulose acetate based system

The rational fixed dose combination of nimesulide (NIM) and tizanidine (TIZ) are available in the market for the relief of inflammations and spasticity as they fall in the similar range of pharmacokinetic profile. The aim of this study was to develop a system which is able to maintain plasma concentration without the need of frequent dosing and less side effects unlike in case of conventional dosage form and applicable for long term therapy.

Bi-layered tablets of nimesulide NIM and TIZ were prepared by using different proportions of cellulose acetate (CA) as the retardant material. Nine formulations of bi-layered tablets i.e., F1 to F9 having different proportion of microparticles developed by varied proportions of polymer: drug ratio i.e., 1:9 -1:3 for NIM and 1:1 – 3:1 for TIZ. The tablets contain microparticle
equivalent to 150 mg of NIM and 6 mg of TIZ as maintenance dose. The equivalent quantity of nimesulide and tizanidine were calculated on the basis of entrapment efficiency of NIM and TIZ microparticles. Sustained release non-disintegrating tablets of NIM and TIZ microparticles were prepared by direct compression of different concentration of microparticles of NIM and TIZ separately. Then these non-disintegrating tablets were further double compressed with loading dose granules equivalent to 50 mg of NIM prepared by melt techniques using excipients lactose, PEG-4000, potato starch, talc and magnesium stearate. The tableting properties of all the granules were studied prior to compression. Each tablet contained equivalent to 200 mg of NIM and 6mg of TIZ.

Physical characteristics and in-vitro release pattern of microparticles and its tablets were studied by using USP dissolution apparatus (USP XXVI/NF21, 2003) type 2 (paddle method) in simulated gastric fluid pH 2.0 and phosphate buffer pH 6.8. The drug content and drug release were found to be calculated by using developed simultaneous determination method of nimesulide and tizanidine hydrochloride by spectrophotometry and reverse phase high performance liquid chromatography. The prepared microparticles were white, free flowing and spherical in shape (SEM study), with the particle size varying from 68.8±1.45 to 87.33±1.06 µm and 175.92± 9.82 to 194.94±14.28µm for NIM and TIZ respectively. Various kinetic models viz., Higuchi and Korsmeyer-Peppas and first order were applied to know drug release pattern data, then found that both drugs microparticles and its tablet formulation (F1-F9) follows diffusion limited drug release pattern. The more
than 85.0 % of NIM and 90.0 % of TIZ were released within 10.0-24.0 h for NIM and 14.0-24.0 h for TIZ, which may be extended due to the microparticulate matrix tablets formulation. To observe the kinetic of drug release, the logarithm of percent drug retained in formulations verses time was plotted and found straight line ($r^2$ values 0.9661-0.9808 and 0.8850-0.9541 for NIM and TIZ respectively). It indicates that all formulations follows first order release rate kinetic pattern. The first order rate constant $K_1$ of formulation F1 to F9 were found to be in the range of K1=0.125-0.286 and 0.083-0.189 %hr-1 for NIM and TIZ respectively. The dissolution data of all formulations were fitted in Higuchi’s model and found, that was linear ($r^2$ values 0.9380-0.9901 and 0.9474-0.9858 for NIM and TIZ respectively). The release mechanism and the release rate constants (% h-1) for NIM and TIZ release from formulations were further elucidated by the power equation i.e., Korsmeyer-Peppas and the value of exponent coefficient (n) were found to be in the range of 0.7359-0.9682 and 0.911-1.0952 for NIM and TIZ respectively indicates anomalous to non anomalous transport type of diffusions among different formulations. The multi-component formulation containing 1:6 (CA: NIM) and 1:1(CA: TIZ) "i.e.," F4 formulation showed more or less similar release time.

All formulations were found to be stable under normal and accelerated stability conditions.

**Conclusion**

The fixed dose combination of nimesulide (NIM) and tizanidine hydrochloride (TIZ) could be prepared by using multi-component microparticulate system
for the relief of inflammations and spasticity. The multi-component tablets containing NIM (1:6) and TIZ (1:1) i.e., formulation F4 showed almost identical release kinetics and it is expected that the time course of action of the two drugs might be similar in biological system, which is an important criterion for the possibility of a rational fixed-dose-combination. Thus designing of multi-component sustained delivery of nimesulide with tizanidine hydrochloride in proper dosing services will minimize the drug toxicity, reduce the overdosing or drug complications, keep health care at minimum cost and ultimately increase the patient compliance.

6.3.2 Ethylcellulose based system
Bi-layered tablets of nimesulide (NIM) and tizanidine hydrochloride (TIZ) were prepared by using different proportions of ethylcellulose (EC) as the retardant material. Nine formulations of bi-layered tablets i.e., F1 to F9 having different proportion of microparticles developed by varied proportions of polymer: drug ratio i.e., 1:9 -1:3 for NIM and 1:1 – 3:1 for TIZ. The tablets contain micro-particles equivalent to 150 mg of NIM and 6 mg of TIZ as maintenance dose. The equivalent quantity of nimesulide and tizanidine were calculated on the basis of entrapment efficiency of NIM and TIZ microparticles. Sustained release non-disintegrating tablets of NIM and TIZ microparticles were prepared by direct compression of different concentration of microparticles of NIM and TIZ separately. Then these non-disintegrating tablets were further double compressed with loading dose granules equivalent to 50 mg of NIM prepared by melt techniques using excipients lactose, PEG-4000, potato starch, talc and magnesium stearate.
The tableting properties of all the granules were studied prior to compression. Each tablet contained equivalent to 200 mg of NIM and 6 mg of TIZ.

Physical characteristics and in-vitro release pattern of microparticles and its tablets were studied by using USP dissolution apparatus (USP XXVI/NF21, 2003) type 2 (paddle method) in simulated gastric fluid pH 2.0 and phosphate buffer pH 6.8. The drug content and drug release were found to be calculated by using developed simultaneous determination method of nimesulide and tizanidine hydrochloride by spectrophotometry and reverse phase high performance liquid chromatography. Physical appearance, hardness, friability, wt. variation and drug content uniformity of all tablet formulations (F1 – F9) were found to be satisfactory. Tablet hardness varied between 5.4±0.7 to 5.6±0.6 kg/cm2 and friability was less than 0.5 % (w/w). The manufactured tablets showed low wt variation "ie," 2.9±0.16 (mean± SD) and a high degree of drug content uniformity "i.e.," NIM 198.32±0.4 mg/tab and TIZ 5.81±0.12 mg/tablet. Various kinetic models viz., Higuchi and Korsmeyer-Peppas and first order were applied to know drug release pattern data.

To observe the kinetic of drug release, the logarithm of percent drug retained verses time was plotted and found straight line (r² value 0.899 to 0.996) indicating that all formulation (F1-F9) follows first order release rate kinetic pattern and their respective release rate constant were calculated. The dissolution data of formulation F1-F9 were fitted in Higuchi’s model and found linear (r² value, 0.959 to 0.996) indicating diffusion controlled release
from the matrix tablet. The release kinetics of F1-F9 formulation were compared and found that F2 formulation has sustained release with similar release rate ($K_1$= 0.129 and 0.134 % h$^{-1}$ for NIM and TIZ respectively) as compared to other formulations. The dissolution data of NIM and TIZ tablets were also fitted to the well-known exponential equation (Korsmeyer equation), which is often used to describe the drug release behavior from polymeric systems. All tablet formulations (F1-F9), the values of $n$ and the Logk for NIM and TIZ ranged from 0.692 - 0.852 and 0.822 - 0.965 and 0.740 - 1.06 and 0.614 – 0.998 respectively, indicating a release mechanism non-fickian (anomalous) for the nimesulide release and non-fickian (anomalous) release to super case-II transport in case of tizanidine. All formulations were found to be stable under normal and accelerated stability conditions.

**Conclusion**

The fixed dose combination of nimesulide (NIM) and tizanidine (TIZ) could be prepared by using multi-component microparticulate system for the relief of inflammations and spasticity. The multi-component tablets containing NIM (1:9) and TIZ (2:1) "i.e.," formulation F2 showed almost identical release kinetics and it is expected that the time course of action of the two drugs might be similar in biological system, which is an important criterion for the possibility of a rational fixed-dose-combination. Thus designing of multi-component sustained delivery of nimesulide with tizanidine in proper dosing services will minimize the drug toxicity, reduce the overdosing or drug
complications, keep health care at minimum cost and ultimately increase the patient compliance.

6.3.3 Development of analytical methods

6.3.3.1 Spectrophotometry

The combination of nimesulide and tizanidine hydrochloride has been emerged as one of the widely prescribed combination in single dosage forms for anti-inflammatory and muscle relaxant. The nimesulide is anti-inflammatory agents in combination with muscle relaxant tizanidine hydrochloride. The literature revealed that no method of simultaneous estimation by UV-spectrophotometer of both the drugs in tablet dosage forms have been reported till date. Hence a simple, rapid, accurate, economical and sensitive spectrophotometric simultaneous method, which is based on the additivity of absorbance for the determination of nimesulide and tizanidine hydrochloride in tablet formulation, has been developed. The Shimadzu Pharmaspec 1700 UV-Visible spectrophotometer with a matched pair of 10 mm quartz cells was used for the experimental purpose. The absorption maxima found to be at 390.5 nm and 319.5 nm respectively for nimesulide and tizanidine hydrochloride in methanol. Nimesulide obeyed the Beer Lambert’s law in the concentration range 5-35µg/ml and tizanidine hydrochloride 5-30 µg/ml. The method was employed for the estimation of drugs in two marketed tablet formulations. The result showed the close proximity to the percentage of label claim. The method was validated with
the recovery study and the result was reproducible. No interference was observed with excipients. The method was found to be accurate, simple and rapid, for routine simultaneous analysis of the tablet formulation without prior separation.

6.3.3.2 Reverse phase high performance liquid chromatography

Both nimesulide and tizanidine hydrochloride are soluble in 0.02 M sodium acetate buffer and acetonitrile in the proportion of 60:40 with apparent pH at 4.0 therefore this composition was selected as solvent. The mobile phase could resolve nimesulide and tizanidine, with better resolution. The retention times of nimesulide and tizanidine are 7.9 min and 5.9 min, respectively. Linearity range for nimesulide and tizanidine were 60-140 µg/ml (r=0.9974) and 1.2-2.8 µg/ml (r=0.9994), respectively. The UV detection was carried out at 320 nm as nimesulide and tizanidine both shows λmax around that wavelength. The UV absorption of tizanidine is much more than nimesulide at this wavelength and because which it was possible to have appreciable response of tizanidine (2µg/ml) and also detector response was not saturated for nimesulide (100 µg/ml) peak.

System precision and assay precision experiment yielded results that were precise with percent relative standard deviation less than 2.0%. Linearity experiment was performed thrice for both the drugs and response was found to be linear in the range of 1.2 to 2.8 µg/ml of tizanidine and 60-140 µg/ml for nimesulide. Regression lined obtained at 95% confidence interval using least square method. Correlation coefficient ‘r’ values (n=3) for both tizanidine and nimesulide were ≥0.999 and % relative standard deviations of slope values
were 1.42 and 0.34 for tizanidine and nimesulide, respectively. The response of both the drugs was linear in the said range. Recovery study was carried out at three levels. Standard drug solutions containing drugs in the range of 10-30 of nominal concentration (2µg/ml for tizanidine and 100 µg/ml for nimesulide) was added to previously analyzed tablet sample solution (test solution). The amount of drug recovered at each level (n=3) was determined. Percentage recovery at each level was calculated. The average recovery obtained for tizanidine and nimesulide were 99.56% and 100.11%, respectively.

Based on validation study data it can be concluded that the proposed method is accurate and precise for the analysis of both drugs. No interference was found form excipients used in tablet formulation and hence the method is suitable for analysis of tablet formulation.

6.4 Conclusion

This novel approach employs combination of two drugs with efficient and safe therapy. In such proposed formulation total amount of drug is reducing which results highly minimization of side effect and improving patient compliance regarding frequent dosing and efficacy. In order to industrial implementation of this technology; it is free from extra burden to company with respect to equipment and machineries needed. The novel technique is proposing the combination of nimesulide/tizanidine hydrochloride and aceclofenac/ tizanidine hydrochloride which is already in combination in conventional dosage form prescribed by physician up to 90% in community,
but here this technology providing long term therapy with efficacy and patient compliance.

Finally, the future will also see the increasing application of multi-component therapy as a front-line defense against the disease, aiming at long-term corrective treatment through the designing of multi-component novel drug delivery system. Thus designing of such proper dosing services will minimizes the drug toxicity, reduces the overdosing or drug complications, keep health care at minimum cost and ultimately increase the patient compliance.
List of Publications

Research papers published


9. Reverse Phase High Performance Liquid Chromatographic Determination of Tizanidine Hydrochloride in Tablet Dosage Form, Biosciences, Biotechnology Research Asia, (In-press)

Research papers communicated


**Review articles published**


**Review article communicated**


**Conferences**

1. Microencapsulated matrix tablet for the sustained release of Dual drug Combination". At IIT New Delhi (Biohorizone2005)

2. Effect of processing variables on the release of microparticulate system intended for sustained delivery of aceclofenac. At 10th APTI National convention, 2005, Nagpur (M.H.)

3. Development and characterization of multi-component microparticulate
system of nimesulide and tizanidine. Accepted for 2nd international symposium on drug discovery and process research to be held on Feb.10-12, 2006, Belgaum, INDIA.

4. Development and characterization of bi-layered multi-component system of nimesulide and tizanidine. 9th International Symposium on pharmaceutical Sciences, Canada, May, 2006. (Accepted)

