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Aims and Scope of Acta Pharmaceutica Sciencia

Acta Pharmaceutica Sciencia is a continuation of the former "Eczacılık Bülteni" which was first published in 1953 by Prof. Dr. Kasım Cemal GÜVEN's editorship. At that time, "Eczacılık Bülteni" hosted scientific papers from School of Medicine-Pharmacy Branch, Istanbul University, Turkey.

Starting from 1984, the name of the journal was changed to "Acta Pharmaceutica Turcica" and became a journal for national and international manuscripts, in all fields of the pharmaceutical sciences in both English and Turkish. (1984-1995, edited by Prof. Dr. Kasım Cemal GÜVEN, 1995-2001, edited by Prof. Dr. Erden GÜLER, 2002-2011, edited by Prof. Dr. Kasım Cemal GÜVEN)

Since 2006, the journal has been publishing only in English with the name, "Acta Pharmaceutica Sciencia" that represents internationally excepted high level scientific standards.

The journal has been publishing quarterly per year except an interval from 2002 to 2009 which released its issues trimestral in a year. Publication was discontinued from the end of 2011.

With this issue in 2017, Acta Pharmaceutica Sciencia is continuing publication with the reestablished Editorial Board and also with support of you as precious scientists.

Yours Faithfully

Prof. Dr. Şeref DEMİRAYAK
Editor

ABDiİBRAHİM

Big Trouble of Small Teeth

Apply it confidently, let him always laugh...

"#1 Teething Gel in Turkey[1]"

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Evaluation of Rational Antibiotic Dispensing in the Community Pharmacy Setting: A Simulated Patient Study

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ABSTRACT

In the present study, it is aimed to evaluate rational antibiotic dispensing without prescription in the community pharmacy setting by using a simulated patient method. This study was conducted over a total of 70 pharmacies in Malatya, located in the east part of Turkey. The person, who acts the husband of a patient with acute uncomplicated rhinosinusitis, visited the pharmacies to conduct the simulated patient scenario. Of the total community pharmacies that were visited 55.7% of them were run by female pharmacists. Thirty-two (45.7%) pharmacists recommended various medication regimens, including antibiotics. Of them, 67.1% referred the simulated patient to a physician. In conclusion, it was observed that dispensing antibiotics without prescription was still high, pharmacists did not take comprehensive medical or medication history from patients, and pharmacists provided insufficient medication information to the patient regarding suggested medications at community pharmacy setting.

Keywords: Antibiotic, Community Pharmacy Services; A Simulated Patient; Pharmacist

INTRODUCTION

The irrational utilization of antibiotics is still a serious global problem which, if one takes into consideration the magnitude of antibiotic resistance, threatens both public health and the economy. In Turkey, the high rate of irrational antibiotic dispensing is a well-recognized fact that has existed for many years in the community pharmacy setting. In Turkey today, it is illegal to dispense antibiotics without prescription. Since 2014, the control of antibiotic dispensing has increased in the community pharmacy setting. However, it is more common to use simulated patient techniques to control antibiotic dispensing in countries in which the rate of antibiotics being dispensed without prescription is high.[1-3]

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The reason for selecting this method is not to audit or supervise the pharmacist. It has been well documented that the most efficient and reliable feedback which can contribute to the development of professional skills can be obtained by using the simulated patient method.[1] It is easier to observe when pharmacists inappropriately dispense antibiotics without prescription by using the simulated patient method.[4]

It has been well documented that in cases where there is no great risk of bacterial infection, treatments with antibiotics do not provide additional benefits. Treatment with antibiotics is not recommended in cases where the duration of symptoms is less than two days and where there is no high fever.[5] However, in many simulated patient studies, it has been observed that antibiotics which were suggested without prescription were common in cases with acute uncomplicated rhinosinusitis or other acute infections.[5-8]

According to the report by the Turkish Ministry of Health based on records of Prescription Information System in 2011 and 2012, it was determined that the rate of prescribing antibiotics by general practitioners were approximately 35.0% and 34.0%, respectively.[9] The elevated utilization of antibiotics is not unique to Turkey and other developing countries; it is also common in Europe. For example, between 1980 and 1990, the rate of increase in antibiotic utilization for upper respiratory infections was 46.0% in the UK, while in France 86.0% in adults and 15.0% for children. It should be emphasised that this increase in the utilization of antibiotics was more prominent for new antibiotics on the market.[8]

It is well known that the rate of antibiotic utilization and prescription is particularly high in cities located in the east and southeast of Turkey.[9] With this information it is hoped that by using a simulated patient method the possible rate of antibiotic dispensing without prescription can be evaluated. The second aim of this present study is to assess the practices of community pharmacists during the recording of patient histories and patient education.

METHODOLOGY

Study Design

This study was conducted in Malatya, a city located in the eastern part of Turkey. The ethical approval for the present study was given by Marmara University, Ethical Committee of Health Science (Protocol Number: 24.12.2014-6). Permission was granted by Malatya Chamber of Pharmacy. After receiving the necessary permission, all the pharmacies located in Malatya were informed about the present study, the aim of which was to evaluate rational drug utilization. After this stage, those pharmacies which did not want to participate in the study were excluded.

Sample Size Calculation

As of December 2014, a total of 214 pharmacies was registered with the Malatya Chamber of Pharmacy. The sample size was sixty-seven pharmacies, with a confidence interval of 95% and error of margin of 10%. The study was conducted over a total of 70 pharmacies. All the pharmacies were listed alphabetically and were randomly selected and allocated random numbers by a computer-based program.

Data Collection

All appointments were carried out with the pharmacists. When the simulated patient entered the pharmacy, he would first ask to talk to the pharmacist. The patient could confirm whether they were talking to the pharmacist by checking the photograph of the pharmacist on the wall; it is law in Turkey that a photograph of the pharmacist be hung on the wall. Although, the information regarding simulated patient was not given consecutively, the simulated patient provided other information if the pharmacist asked for it.

The simulated patient visited the community pharmacies as a husband of patient with acute uncomplicated rhinosinusitis. The simulated patient was trained regarding the standard information to be provided by the researchers and informed about the privacy of all information that would be gathered during the present study.

The scenario for the simulated patient was created according to previously performed studies.[5-8] A simulated patient demanded medication for pain located in the region of the frontal sinuses. The other information regarding the simulated patient was listed as following; he was purchasing this medication for his wife, who was 24 years old; she had a fever of 38-38.5^{o}C, she also had running nose; she had a history of rhinosinusitis and she had used antibiotics in the past, but she could not remember the name of the antibiotic; she was currently only using oral contraception and had no history of allergies. If the pharmacists could not give any information regarding suggested medication, the simulated patient would ask information regarding side-effects of the suggested medication. If the pharmacist referred a simulated patient directly to a doctor, a simulated patient would inform the pharmacist that they had an appointment for tomorrow, but they wanted the pharmacist to advise on what to do until that appointment.

After each community pharmacy was visited, the simulated patient filled the check list which had been drawn up for the purpose of the present study. Due to ethical concerns, no audio or video records were used during the study. Any suggested medications were not purchased from the community pharmacy. When

any medication was suggested by the community pharmacists, the simulated patient would reply that he did not have enough money to buy the medication or he already had them at home.

Statistical Analysis

All variables were presented as mean ± standard deviation. Ordinal and nominal data were introduced as number [n] and percentage [%].

RESULTS

The demographic data

Of the total community pharmacies that were visited 55.7% of them had female pharmacists and 44.3% were run by male pharmacists. It was observed that all pharmacists gave less than 3 minutes of attention to the simulated patient. The mean number of questions asked by the pharmacists to the simulated patient was 3.17±1.65. The distribution of the total number of questions asked by community pharmacists is presented in Figure 1.

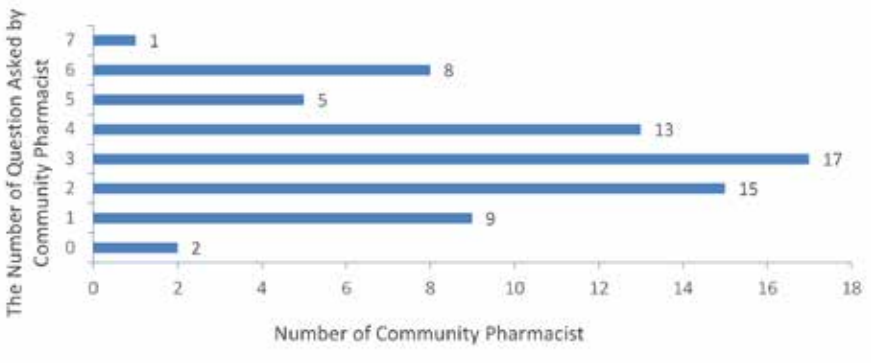

Figure 1: The distribution of the total number of questions asked by the community pharmacist

The attitude of community pharmacists while patient history taken

Of these, 77.1% asked about the age of the patients. Only 25.7% asked for information regarding the duration of the patient's symptoms. Most pharmacists [82.9%] did not ask whether the patient had any chronic disease, while only 15.7% took a history of the patient's medications for chronic illnesses. The symptoms and complaints of simulated patients were investigated by only 18.6% of the pharmacists. Of these, 70.0% asked whether fever was present. How the rhinosinusitis was managed before coming to the community pharmacy was questioned by only 21.4% of the pharmacists. The practice of community pharmacists during the taking of patient history is shown in Table 1.

Table 1: The attitude of community pharmacists while patient history taken

	%
Allergy	0%
Age	77.1%
Symptoms	81.4%
Period of symptoms that patient had	25.7%
Comorbidities	82.9%
Fever	70.0%
Utilization of medication for chronic illnesses	15.7%
Management of rhinosinusitis before coming to the community pharmacy	21.4%

The patient education practices of the community pharmacists

When the medication information that was provided by pharmacists is evaluated, 75.7% of them provided information regarding the reason for using the medication, while 60.0% of them explained how to use the medication. However, only 31.4% clarified when the medication was to be used and only 8.6% of them provided information about how long the medication should be used. None of the community pharmacists provided any information about other medications that could be used if an unusual condition occurred or if the patient forgot to take the medication. The patient education practices of the community pharmacists are presented in Table 2.

Table 2: The patient education practices of the community pharmacists

	%
Indication	75.7
How to use the medication	60.0
When to use the medication	31.4
Duration of medication	8.6
What to do when unusual condition happens	0.0
What to do if he/she forget to take his/her medication	0.0

The suggested medication regimens

Only eleven pharmacists did not suggest any medication to the simulated patient. However, thirty-two (45.7%) pharmacists recommended various medication regimens, including antibiotics. The suggested medication regimens and suggested medications are shown in Table 3 and Table 4, respectively.

Table 3: The suggested medication regimens

	n
No medication	11
Antibiotic + NSAIDs	15
Antibiotic alone	17
NSAIDs alone	25
Combined medications product for cold	2

Table 4: The suggested medications

	n
No Medications	11
Cefuroxime + Dexketoprofen	3
Cefuroxime + Naproxen	2
Cefuroxime	5
Cefuroxime + Diclofenac	1
Naproxen	4
Dexketoprofen	17
Amoxicillin + Clavulanic Acid	12
Amoxicillin + Clavulanic Acid + Dexketoprofen	6
Diclofenac	4
Acetaminophen + Pseudoephedrine + Chlorpheniramine + Oxolamine	1
Amoxicillin + Clavulanic Acid + Naproxen	3
Acetaminophen + Pseudoephedrine + Chlorpheniramine	1

The attitude of community pharmacists

Of these, 67.1% referred the simulated patient to the physician. Among these community pharmacists, 85.1% of them directly referred the simulated patient to the physician and 14.9% of them referred the simulated patient to the physician if no improvement in symptoms should occur.

DISCUSSION

The common usage of antibiotics in the population cannot only be attributed to the prescription rate of physicians. It is well known that despite legal restrictions in many countries, dispensing antibiotics without prescription at community pharmacies and the attitude of the patient have also contributed to an increase in usage of antibiotics.

In the present study, 45.7% of pharmacists recommended various medication regimens, including antibiotics, to simulated patient with symptoms of non-bacterial rhinosinusitis. Of these, 67.1% referred the simulated patient to a physician. Although not purposely timed, the control for dispensing antibiotics without prescription at the community pharmacy had been strictly increased at the time when the present study was conducted. The most striking result of the present study was that after this new implementation, the rate of dispensing antibiotics without prescription was still high.

There are lots of similar studies with similar finding conducted in many different countries. In a systematic review of many studies conducted in various countries between 1970 and 2009[4] it has been determined that the utilization rate of antibiotics without prescription was between 19.0% and 100.0%, except in North America and northern Europe. In the most of the studies that were involved in this systematic review, the utilization of antibiotics without prescription was more common for non-bacterial infections. In this systematic review it was determined that according to the data of studies which used the simulated patient method, antibiotics without prescription were more commonly dispensed in community pharmacies.

In agreement with this present study, in a study conducted in Greece in 2001 it was demonstrated that antibiotics were dispensed without prescription, although in contravention of implementations in the country. In this study, similar to the present study, simulated patients with rhinosinusitis were used. It was observed that 65.0% of the pharmacists suggested broad-spectrum antibiotics to the simulated patients with high fever [40°C] and also 71.0% of them advised broad-spectrum antibiotics to simulated patients with low fever [38.5°C]. In this study, the percentages of simulated patients referred to physicians by pharmacists was 57.0% for simulated patients with high fever [40°C] and 71.0% for simulated patients with low fever [38.5°C].[8] When considering the study conducted in Greece[8] and the present study it can be seen that similar results were attained, despite a period of almost fifteen years between the two studies.

In another study conducted with the simulated patient method for patients with non-complicated rhinosinusitis in Brazil, it was concluded that the percentage of dispensing antibiotics at community pharmacies was 58.0%.[5] Contrary to expectations, it was also determined that dispensing antibiotics to simulated patients with non-complicated rhinosinusitis by pharmacists was greater when compared with pharmacy technicians.[5]

In a simulated patient study with complaints about various acute infections conducted in Saudi Arabia, the percentage of antibiotics being dispensed without prescription was 77.6% for 367 pharmacies.[7] In another study conducted in the

United Arab Emirates, the percentage of antibiotics dispensed without prescription was 68.4% in the community pharmacy setting.[10] In this study, the antibiotics suggested by community pharmacies was a combination of penicillin including β-lactamase inhibitors, penicillin with extended spectrum and second-generation cephalosporin.[10] In the present study the most commonly suggested antibiotics without prescription were amoxicillin and clavulanic acid and cefuroxime axetil.

In the studies mentioned above and the present study, studies conducted in five different countries, high and similar rates of dispensing antibiotics without prescription were determined. It is well known that the utilization of antibiotics without prescription is common, particularly in developing countries. In a study conducted in the Lao People's Democratic Republic, a developing country, 91.0% of patients said that they had received antibiotics without prescription from a community pharmacy during the previous year and 79.0 of patients stated that they did not used their antibiotics during the recommended period.[11]

In a study conducted in Indonesia in which three different simulated patient scenarios were used in the community pharmacy setting, it was determined that 91.0% of pharmacies dispensed antibiotics without prescription; most also provided no health information unless specifically asked by the simulated patient.[6]

In the studies conducted in these countries, which have low social economic status, it is obvious that the rate of dispensing antibiotics without prescription is higher than the present study or the rate shown in studies conducted in developing countries. One exception is a study conducted in Zimbabwe[12]. Interestingly, in this study, the general rate dispensing of antibiotics without prescription was low.

According to the results of a study which aimed to determine the rate of dispensing antibiotics without prescription in Europe in 2006, it was concluded that the rate in eastern and southern Europe was high when compared to countries in northern and western Europe.[13] Although illegal, the rate of dispensing antibiotics without prescription, determined with a simulated patient method, was 45.0% in Spain.[14] This rate was not as high as that found in other studies or in the present study; moreover, the pharmacists in this study gave better and more elucidatory information to the simulated patient. In another study conducted in Spain[15], 28.0% of the participants declared that they had used antibiotics in the previous 6 months for the common cold or sore throat. Moreover, among participants who had used antibiotics in the previous 6 months, 41.0% mentioned that they used these antibiotics without prescription.[15]

Another situation that should be investigated is the existing discrepancy between real-life applications of pharmacist or pharmacy technicians and the information provided.

Although it is known that a lack of knowledge leads to application errors, it is thought-provoking that the attitude of pharmacists was not in concord with recent pharmacotherapy guidelines, even in cases where standard information is available, such as with upper respiratory tract infections or diarrhoea. In a study conducted in Vietnam a questionnaire and simulated patient method was used; 20.0% of pharmacists declared in the questionnaire that they had dispensed antibiotics. However, it was seen that 83.0% had sold antibiotics to simulated patients in the study. Although 81.0% emphasized the lesser effect of antibiotics when used over a short period in the questionnaire in this study, 48.0% advised simulated patients to use antibiotics for less than five days. The results of this study are a good example of the reliability of simulated patient method when compared to questionnaire studies for obtaining the rate of dispensing antibiotics without prescription, and determining the knowledge and attitude of pharmacists towards acute infections. [16]

When evaluating previous studies and this study it can be seen that the rate of dispensing antibiotics without prescription is still very high. More attention should be given to the high rate of dispensing antibiotics without a prescription when the results of these studies are evaluated. The possibility of preventing the sales of antibiotics without prescription should be investigated. It is important to list previous suggestions and actions for preventing the dispensing of antibiotics without a prescription by giving examples from various countries.

In conclusion, it has been observed that the dispensing of antibiotics without prescription is still high, that pharmacists did not take comprehensive medical or medication histories from the patients, and that pharmacists provided insufficient medication information to the patient regarding suggested medications in the community pharmacy setting. To avoid irrational dispensing of antibiotics, it is essential that both health care providers and the general population be educated. Although dispensing antibiotics without prescription is illegal in some countries in the world, new regulations must be introduced to avoid dispensing antibiotics without prescription.

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Development and In Vitro Characterization of Microemulsions of Isotretinoin

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ABSTRACT

Microemulsions are nano-sized colloidal drug carriers which offer several advantages such as ease of preparation, thermodynamic stability, high solubilizing capacity for both of lipophilic and hydrophilic drugs and penetration enhancement. The aim of this study was to prepare novel microemulsions of isotretinoin, a highly lipophilic anti-acne drug, for its topical application. The *pseudo-ternary* phase diagrams were constructed at different oil to surfactant/co-surfactant mixture using isopropyl myristate (oil phase), Labrasol (surfactant), Kolliphor HS15, Kolliphor EL or Plurol Oleique CC497 (co-surfactant) and water. The physicochemical properties and storage stability of microemulsions were investigated. The developed microemulsions were characterized in terms of isotropy, particle size and size distribution, pH, refractive index, rheological behaviour, and conductivity. Spherical shape and droplet size of microemulsions were supported by transmission electron microscopy (TEM). Optimized formulations were found to be physically stable over a period of six months. In conclusion, microemulsions could be promising colloidal carriers for topical delivery of isotretinoin.

Keywords: microemulsion, colloidal systems, isotretinoin, topical drug delivery

INTRODUCTION

There has been increased interest during recent years in the use of topical vehicles that may modify drug penetration into the skin[1].The most difficult aspect in skin delivery of drugs is to overcome the barrier of *stratum corneum*. Various strategies have been employed to achieve delivery of drugs into skin. Among these strategies, microemulsions have been suggested to serve as efficient promoters of drug localization to skin[2-4].

Microemulsions are thermodynamically stable, fluid and isotropic colloidal nanocarriers with a dynamic microstructure that form spontaneously by combin-

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ing appropriate amounts of oil, water, surfactant and a co-surfactant[5-7]. They are prepared mostly by the phase titration method and can be depicted with the help of *pseudo-ternary* phase diagrams. Some of the potential mechanisms by which microemulsions would improve transport of drugs to the skin are described below:[1,3,7,8-10]

– Ingredients of microemulsions can modify the diffusional barrier of the *stratum corneum* either by perturbation/fluidization of intercellular lipid bilayers or denaturation of intracellular keratin or modification of its confirmation.

– Due to the high solubilization capacity of microemulsions, both for the hydrophilic and lipophilic drugs, an increased concentration gradient towards the skin can be reached.

– The ultralow interfacial tension and the continuously fluctuating interfaces of microemulsions can facilitate drug penetration into deeper skin layers compared to conventional formulations.

– The partitioning and solubility of drugs in *stratum corneum* could be increased depending on microemulsion composition.

– The internal phase can act as a drug reservoir resulting controlled and sustained release from microemulsions.

In view of all these features of microemulsions, the present study aims to explore microemulsions as alternative topical carriers for isotretinoin, with an objective to facilitate skin targeting of the drug while decreasing its systemic exposure and toxicity. For that purpose the *pseudo-ternary* phase diagrams of microemulsion systems were constructed at different surfactant/co-surfactant ratios using isopropyl myristate (IPM) as oil phase, Labrasol as surfactant and Kolliphor HS15 (KHS), Kolliphor EL (KEL) or Plurol Oleique CC497 (PLO) as co-surfactant. It has been reported that IPM enhances skin permeation by acting as a fluidizer of intercellular lipids and affects the lipid rich phase in the stratum corneum, thereby reducing its barrier function[11]. Labrasol is a surfactant which has been shown to significantly enhance the permeation of lipophilic drugs through the skin[12]. The co-surfactants KHS, KEL and PLO served frequently as penetration enhancers in the scientific literature[13-17]. The physicochemical properties such as droplet size, refractive index, electrical conductivity, pH, and rheology of the microemulsions were measured and TEM analysis was performed. Optimized formulations were found to be stable over a period of six months at 25°C ±2°C and 60%±5% relative humidity (RH).

METHODOLOGY

Materials

Isotretinoin, polyoxyl 35 castor oil (KEL) and polyoxyl 15 hydroxystearate (KHS) were kind gifts of BASF (Limburgerhof, Germany). IPM was purchased from Sigma (St. Louis, MO, USA). Caprylocaproyl macrogol-8 glycerides (Labrasol) and polyglyceryl-3 oleate (PLO) were kindly provided by Gattefossé (Lyon, France). All other chemicals and reagents used were of analytical grade.

Construction of Pseudo-ternary Phase Diagrams

Pseudo-ternary phase diagrams were constructed to determine the appropriate concentration range of components necessary for the formation of microemulsions prepared with the water titration method at ambient temperature. IPM (oil phase) to surfactant/co-surfactant mixture ratio varied from 1:9 to 9:1 (w/w). Based on pre-formulation study data, the mixing ratios of surfactant/co-surfactant (K_m) were fixed as 4:1 and 3:1. The mixture of oil and surfactant/co-surfactant at predetermined weight was titrated drop wise with water under moderate magnetic stirring, at ambient temperature. Following each addition, the mixtures were stirred and then allowed to equilibrate. After equilibration, they were visually assessed for phase separation, transparency and flow properties. Transparent, homogenous (single-phase) and, low viscous systems were considered as microemulsion[18]. Titration was stopped with the presence of a cloudy system and/or phase separation. The quantity of the aqueous phase required to make the mixture turbid was recorded. Based on the phase diagrams, appropriate concentration of components were chosen and used in the preparation of drug loaded microemulsions. Drug loaded microemulsions were prepared as follows: Isotretinoin (0.05%) was weighed into a small glass vial and dissolved in required quantity of IPM under magnetic stirring. Appropriate amount of surfactant/co-surfactant was added to oil phase and was mixed to yield a homogenous solution. The solution was titrated with water up to 100% (w/w) under magnetic stirring and the obtained microemulsion systems were allowed to equilibrate at ambient temperature. Drug loaded microemulsions were stored in well closed amber coloured vials at room temperature, and protected from light due to the very poor photostability of isotretinoin.

Characterization of Microemulsions

Droplet Size Measurements

The droplet size and polydispersity index (PDI) values of plain and isotretinoin loaded microemulsions were determined at 25°C with permanent angle of 173° by a Zeta Sizer (Nano ZS, Malvern Instruments, UK) without dilution with water

to avoid phase separation[17]. All samples were analyzed in triplicates after prefiltering (0.45 mm, Millex, Merck Millipore, Billerica, MA, USA). The droplet size was expressed as average size of droplets in the system and PDI indicated the width of the size distribution[19].

Microscopic Analysis

Polarized Light Microscopy

Microemulsion formulations were examined under a polarized light microscope (Olympus BX51 U-AN 360, Tokyo, Japan) in order to verify their isotropic nature. A drop of the freshly prepared microemulsion was placed between a coverslip and a glass slide and observed under cross-polarized light. It is expected that an isotropic material, such as a microemulsion, will not interfere with the polarized light and the field of view will remain dark[20].

Transmission Electron Microscopy

Transmission electron microscopy (TEM) was used to characterize the morphology of the microemulsions[21]. For this purpose, a microemulsion drop was directly deposited on a carbon-coated copper grid and allowed to dry for 60 min at room temperature. Then, the grid stained with one drop of 2 % (w/w) phosphotungstic acid, excess of the solution was removed with a filter paper and allowed to dry for 5 min before examination under the electron microscope (JEM-1011, JEOL, Japan).

Electrical Conductivity Measurements

The electrical conductivity of plain and drug loaded microemulsions were measured with a conductometer (EuTech PC 700; Eutech Instruments, Landsmeer, the Netherlands) at room temperature.

For the assessment of the microstructure 3 mL of the IPM/(Labrasol/KEL) mixture at ratio 1:9 was titrated by water stepwise and at each step, 1 mL of sample was used for the measurement of the electrical conductivity at room temperature[22]. The evaluation was made by plotting the conductivity values (κ) versus the water percentages (φ_w) obtained experimentally and the percolation thresholds were determined from the peaks of the plot. The measurements were carried out in triplicate, and results were presented as mean ± SD.

Rheology

Rheological measurements (shear stress, shear rate and apparent viscosity) were performed using a cone and plate Brookfield Rheometer (Brookfield DV3THACJ0, Middleboro, MA, USA) in triplicate in a temperature controlled environment at 25°C and rotational speed was ranged from 10-100 rpm.

pH

The pH values of plain and drug loaded microemulsions were measured by direct immersion of pH meter electrode (EuTech PC 700; Eutech Instruments, Landsmeer, the Netherlands) in the formulations at room temperature. Before each measurement calibration was performed using standard buffer solutions of pH 4.0, 7.0, and 10.0, respectively. The measurements were carried out in triplicate, and results were presented as mean ± SD.

Refractive Index

The refractive index values of the plain and drug loaded microemulsion formulations were measured by an Abbe refractometer (Atogo Co., Ltd, Tokyo, Japan) by placing one drop of the microemulsion sample on the slide at room temperature. The measurements were carried out in triplicate at 25°C.

Evaluation of Stability

Centrifugation

Microemulsions were centrifuged (Hettich Zentrifügen D-7200) at 15000 rpm for 30 min at ambient temperature to assess the thermodynamic stability. The formulations that did not show any phase separation and cloudy appearance after centrifugation were taken for freeze-thaw cycle.

Freeze-thaw cycle

Freeze–thaw cycles were performed by freezing the microemulsions at −20◦C for 12h followed by thawing at 25◦C for 12h. This process was repeated two times for each sample. Then the samples were examined for clarity, phase separation and droplet size.

Storage stability

The storage stability of plain microemulsions was followed according to ICH Q1 (R2) at 25±2°C and 60%±5% relative humidity (RH) up to 6 months[23]. The physicochemical parameters include appearance and droplet size and its distribution were determined.

Statistical Analysis

The statistical analysis was performed using one-way analysis of variance. A multiple comparison test was used to compare different microemulsion formulations and $p<0.05$ was considered as level of significance (GraphPad Prism Software, La Jolla, CA, USA).

RESULTS AND DISCUSSION

The main goal of the topical therapy is to target the drug to viable epidermis and upper dermis, by minimizing systemic absorption. However, due to its high lipophilic character (logP: 5.01), isotretinoin tends to accumulate on the skin surface and in the upper *stratum corneum*, thus its penetration into the lower layers is limited, which restricts the efficiency of topical treatment[24]. Nano-sized colloidal carriers such as microemulsions are considered appropriate carriers due to the increment of partitioning and solubility of drug in *stratum corneum,* enhancement of thermodynamic activity of drug in the vehicle and/or increasing the permeability of skin[8,25]. Most of the studies demonstrate that more pronounced drug deposition in skin layers rather than percutaneous permeation can be obtained with microemulsions[3,4,9]. Taking all these into consideration, microemulsion type colloidal carriers of isotretinoin were developed and characterized with the aim to increase the dermal penetration of the drug.

Construction of Pseudo-ternary Phase Diagrams

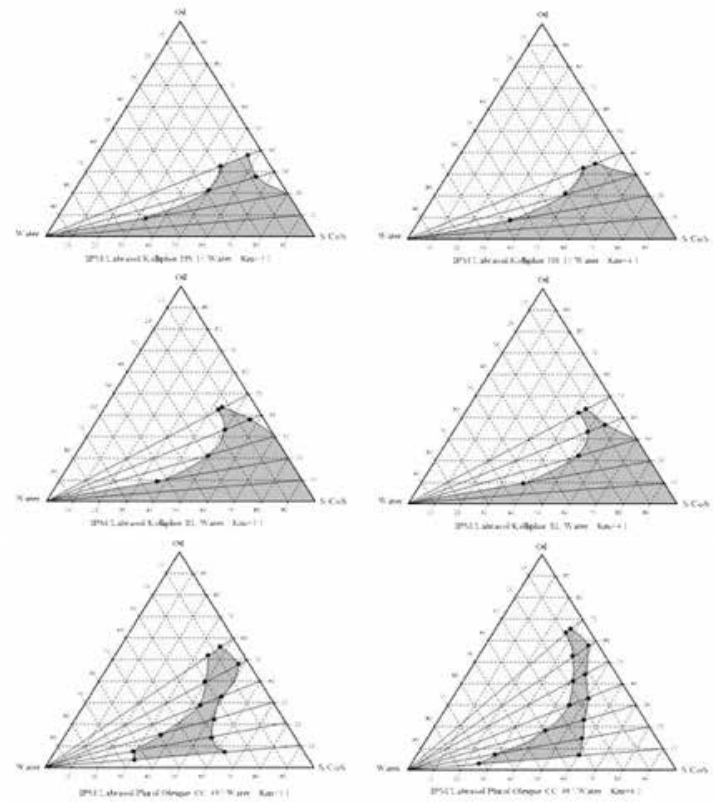

Figure 1: *Pseudo-ternary* phase diagrams of microemulsion systems containing IPM as oil, Labrasol as surfactant, Kolliphor HS 15 or Kolliphor EL or Plurol Oleique as cosurfactant for Km=3:1 and Km=4:1

The microemulsion region can be shown graphically in *pseudo-ternary* phase diagrams, as ratios between oil, water and a fixed mixture of surfactant/co-curfactant[26]. The phase diagrams of the prepared microemulsions with K_m 4:1 and 3:1 are shown in Fig. 1. Water titration method was used to obtain the components and their concentration ranges that can result in large existence area of microemulsion[19,27]. A large microemulsion area in the phase diagram is usually attributed to the progressive reduction of the interfacial tension and indicates the positive effect of surfactant and co-surfactant on the phase properties[18].

The water dilution lines representing an increase of water content while decreasing oil, surfactant and co-surfactant levels were plotted on the phase diagrams. The shaded areas were identified as microemulsion areas and the remaining region of the phase diagram represents turbid and conventional emulsions based on visual observation. The area of isotropic microemulsion region changed slightly in size with increasing ratio of surfactant/co-surfactant. The composition of the prepared microemulsion systems consist of IPM (oil phase), Labrasol (surfactant) and KHS, KEL or PLO (co-surfactant) is given in Table 1.

Table 1: Composition of the optimized microemulsion formulations.

CODE	S:CoS	IPM (%)	Labrasol (%)	KHS (%)	KEL (%)	PLO (%)	Water (%)
ME-KHS1	3:1	5.50	37.50	12.50			44.44
ME-KHS2	3:1	3.80	26.25	8.75			61.14
ME-KHS3	4:1	5.50	40	10			44.44
ME-KHS4	4:1	3.80	28	7			61.14
ME-KEL1	3:1	5.50	37.50		12.50		44.44
ME-KEL2	3:1	3.80	26.25		8.75		61.14
ME-KEL3	4:1	5.50	40		10		44.44
ME-KEL4	4:1	3.80	28		7		61.14
ME-PLO1	3:1	6.50	43.88			14.63	34.94
ME-PLO2	3:1	5.50	37.50			12.50	44.44
ME-PLO3	4:1	6.50	46.80			11.70	34.94
ME-PLO4	4:1	5.50	40			10	44.44

S: Surfactant, CoS: Co-Surfactant

Depending on the physicochemical properties of a drug, different types of microemulsions can be the optimal carrier. Therefore, it is necessary to find the appropriate composition and concentration of components to maximize the drug delivery efficacy of microemulsions[9]. The oil phase, surfactant and co-surfactants for developing isotretinoin loaded microemulsions were selected on the basis of the existence of the microemulsion area and water solubilization capacity. IPM

is among the most frequently selected components of the oil phase in microemulsions. Water solubilization capacity of IPM is reported to be the highest among various oils, such as oleic acid, used in microemulsion formulation[28].

In our study, the quantity of isotretinoin to be loaded to the microemulsion formulations has been kept equivalent to its commercial topical formulation (Isotrexin Gel, 0.05%) and the choice of oil, surfactant and co-surfactants was based on the ability of these components on a stable, skin compatible microemulsion formation with sufficient water content but rather drug solubility[9,22]. When choosing components for microemulsions, it is also important to balance solubility/permeation properties with toxicological considerations[26]. All of the formulated microemulsions were considered as safe regarding the in vitro cytotoxicity study which has been published previously[24].

Characterization of Microemulsions

Droplet Size Measurements

The effect of microemulsion droplet size and large surface area/volume ratio on drug transport into the skin has been shown with several studies[9]. In our study, the mean droplet diameter of plain and drug loaded microemulsions were found in the range of 1.38±0.01 - 9.49±0.09 nm and 1.40±0.03 - 9.56±0.14 nm, respectively (Fig. 2). There is no significant difference in average droplet size observed after loading isotretinoin (p>0.05). It has been found that increasing water content lead to a decrease in microemulsion droplet size. The formulations containing PLO as co-surfactant (ME-PLO1 - ME-PLO4) presented the highest average droplet size and the droplet diameter increased with the increasing oil and surfactant content. Microemulsion formulations ME-KHS2, ME-KHS4, ME-KEL2 and ME-KEL4 containing KHS and KEL as co-surfactants presented the lowest average droplet size with decreased concentrations of oil and surfactant/co-surfactant mixture.

Polydispersity index (PDI) is an important parameter in the characterization of colloidal drug carriers since it reflects the physical stability of the system and provides information about homogeneity of the samples[29,30]. The PDI values lower than 0.4 indicated homogenous microemulsion systems with narrow size distribution in our study. Incorporation of isotretinoin did not affect the PDI of the microemulsions significantly (p > 0.05).

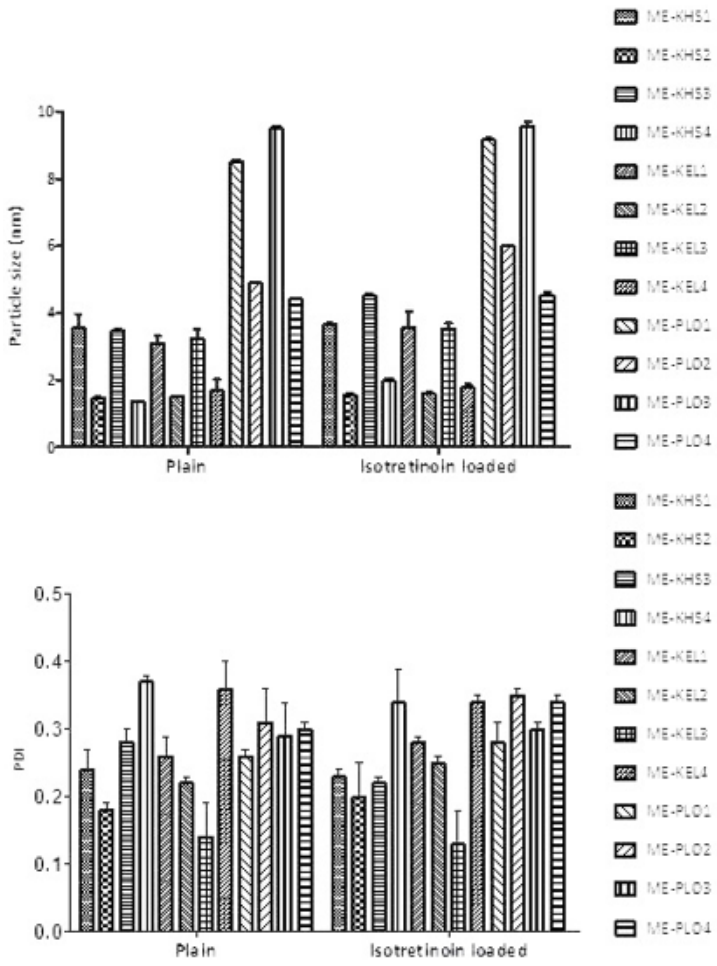

Figure 2: Droplet size and PDI of the plain and isotretinoin loaded microemulsions

Microscopic analysis

It is well known that various structures, such as liquid crystals, can be formed by microemulsion formulation depending on the components and their concentration[17]. Also the incorporation of the drug can affect the microemulsion structure. Cross-polarized light microscopy is a suitable method for differentiating liquid crystals. Under cross-polarized light microscopy, birefringence can be observed for lamellar and hexagonal liquid crystals but no birefringence is observed for microemulsions[31]. In our study, the completely dark appearance under the polarized light microscope confirmed the isotropic nature of the prepared microemulsions. The TEM images of one formulation (ME-KEL3) are shown in Fig. 3, which prove that the microemulsion possessed homogeneous and spherical droplets.

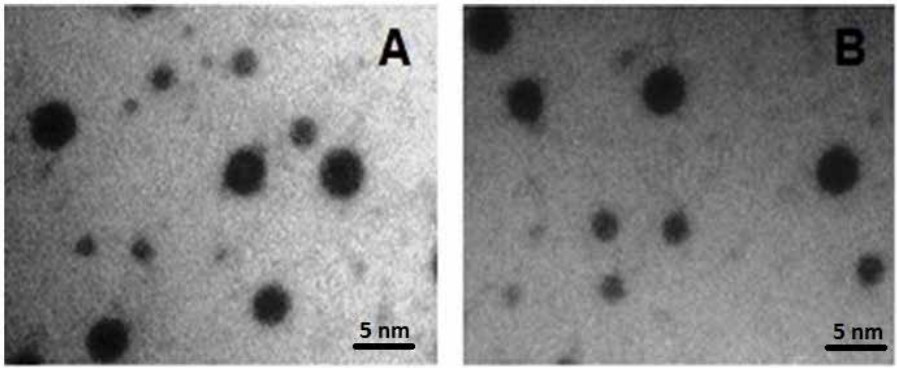

Figure 3: Transmission Electron Microscopy images of A) plain and B) drug loaded microemulsion formulation ME-KEL3

Electrical Conductivity

The electrical conductivity of plain microemulsion formulations was in the range of 7.50±0.06-77.65±0.21 mS/cm (Table 2) and increased by the increasing amount of water. The increase in conductivity might be caused from the increase in dissociation of surfactant (Labrasol) as a function of water content[11, 32].

Table 2: Conductivity, viscosity, pH and refractive index of the plain and isotretinoin loaded microemulsions

Code	Conductivity (µS/cm)		Viscosity		pH		Refractive Index	
	Plain	Drug Loaded	Plain	Drug Loaded	Plain	Drug Loaded	Plain	Drug Loaded
ME-KHS1	50.60±0.04	53.41±0.24	63.79±0.03	64.01±0.01	6.18±0.01	6.51±0.01	1.407±0.002	1.407±0.003
ME-KHS2	77.65±0.21	82.23±0.09	23.96±0.05	24.16±0.02	5.78±0.01	6.04±0.01	1.384±0.002	1.384±0.001
ME-KHS3	40.60±0.07	40.67±0.14	55.05±0.08	56.02±0.04	6.16±0.01	6.32±0.01	1.407±0.002	1.407±0.003
ME-KHS4	68.76±0.14	73.66±0.12	22.15±0.06	22.17±0.07	5.77±0.01	5.85±0.01	1.384±0.001	1.384±0.002
ME-KEL1	37.46±0.05	33.17±0.01	88.00±0.19	87.98±0.21	4.63±0.01	4.84±0.01	1.407±0.005	1.408±0.003
ME-KEL2	71.24±0.08	64.25±0.08	41.22±0.11	42.01±0.21	4.22±0.01	4.35±0.01	1.384±0.001	1.385±0.002
ME-KEL3	34.07±0.05	31.43±0.04	73.88±0.15	74.08±0.18	4.64±0.01	4.87±0.01	1.407±0.001	1.408±0.002
ME-KEL4	63.35±0.12	62.35±0.07	35.78±0.14	35.99±0.05	4.19±0.01	4.38±0.01	1.384±0.001	1.385±0.001
ME-PLO1	7.50±0.06	6.62±0.07	59.17±0.11	59.19±0.11	4.77±0.02	4.90±0.02	1.419±0.003	1.419±0.002
ME-PLO2	15.86±0.03	14.38±0.04	57.20±0.08	57.89±0.10	4.47±0.04	4.68±0.04	1.407±0.001	1.407±0.002
ME-PLO3	9.14±0.04	9.01±0.03	59.66±0.10	60.03±0.06	4.87±0.01	4.91±0.01	1.419±0.002	1.420±0.004
ME-PLO4	18.84±0.06	16.67±0.06	49.88±0.17	51.01±0.12	4.58±0.02	4.68±0.02	1.407±0.002	1.408±0.001

Microemulsions exhibit percolation phenomena at certain volume fractions of water. This is generally accompanied by an increase in the electrical conductivity of microemulsions, which often has been used as a method for internal structure characterization[9,33]. According to the percolation theory, phase transformation from W/O type structure to *bicontinuous* systems and then the formation of O/W type microemulsions occur as aqueous content in the system increases[9,14]. The percolation threshold refers to the critical water volume fraction at which isolated droplets form infinite clusters through the emergence of *bicontinuous* structures[34].

The IPM/(Labrasol/KEL) mixture at the oil: surfactant/co-surfactant ratio of 1:9 and the ratio of Labrasol:KEL (K_m)4:1 as weight could be diluted by water to higher than 98% (w/w) water content and the resulting sample remained as a clear microemulsion. Also, this dilution line included the microemulsion formulation ME-KEL3 which has been presented the highest isotretinoin accumulation in pig skin in our previous study[24]. Therefore, in accordance with the study of Zhang&Michniak-Kohn[22], the microemulsion microstructure was studied along this water dilution line and the measured electrical conductivity values (κ) plotted against the water content (φ_w) as shown in Fig. 4. The κ vs. φ_w curve showed three distinct parts, which could be fitted by linear regressions at low and high aqueous phase regions, corresponding to W/O and O/W microstructures. Based on these results, it can be deduced microstructure transition points from W/O to *bicontinuous* and from *bicontinuous* to O/W were at water content of about 15% and 75%, respectively.

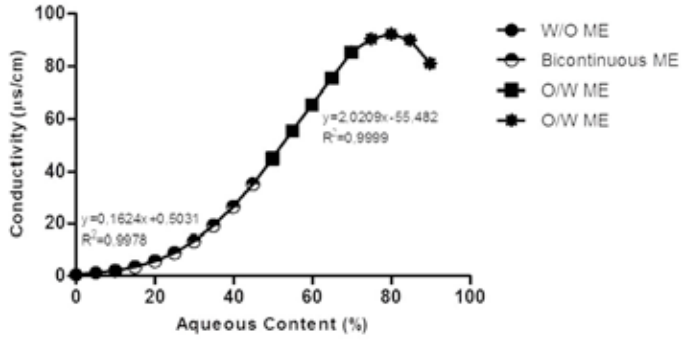

Figure 4: The plot of the microemulsion electrical conductivity to aqueous content

Rheology

The viscosity of a microemulsion is the function of the type of its components (oil, surfactant, co-surfactant and water) and their concentrations[35]. The viscosity of plain microemulsions was in the range of 22.15±0.06 - 88.00±0.19 cPs

(Table 2), and tended to increase as the amount of the oil and surfactant mixture in the formulation increased. This data is in accordance with the literature[11]. The viscosity values of isotretinoin loaded microemulsions were slightly higher than the values of unloaded formulations. All microemulsions exhibited Newtonian flow behavior (Fig. 5) due to their very low viscosity values as expected from microemulsions[10, 15, 17, 31].

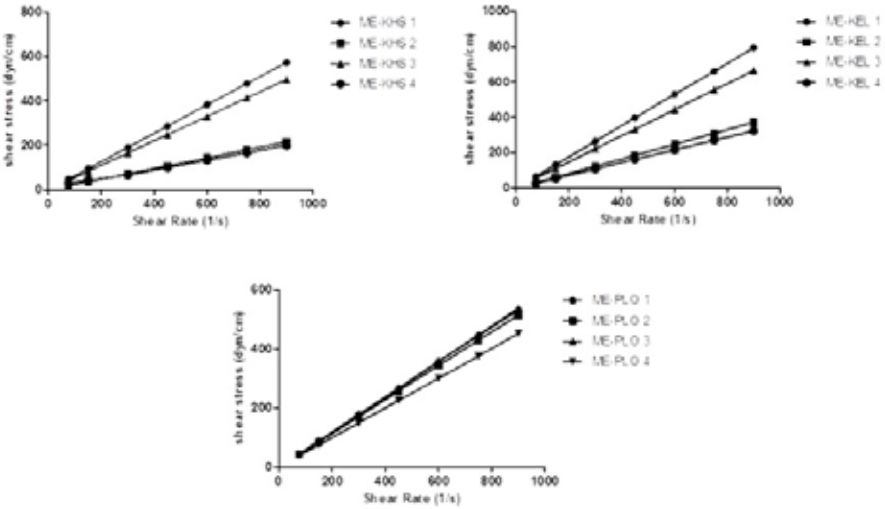

Figure 5: The plotes of shear stress versus shear rate for all of microemulsions prepared

pH

Table 2 shows the physicochemical characteristics of isotretinoin loaded microemulsions and their blank counterparts. The pH of the plain microemulsion formulations were between 4.19±0.01 and 6.18±0.01 (Table 2). Incorporation of isotretinoin slightly increased the pH to the range of 4.35±0.01-6.51±0.01. The pH of microemulsions could be considered as suitable for cutaneous application as it has been reported that pH values in the range of 3 to 10 are tolerable by the skin and do not change the skin penetration of lipophilic substances[36].

Refractive Index

The refractive index provides information about the dispersed and continuous phases of microemulsions and indicates their isotropic nature. The refractive index of microemulsions is expected to be close to the refractive index of the pure component forming the continuous phase[37]. The refractive index values of the plain and drug loaded microemulsions are demonstrated in Table 2. The refractive index values of isotretinoin loaded microemulsions (1.384±0-1.420±0) were similar to their blank counterparts (1.384±0-1.419±0) and confirmed the transparent nature of the formulations[38].

Physical Stability

After centrifugation at 15000 rpm for 30 min microemulsion formulations remained homogenous without any phase change such as turbidity or phase separation. Freeze thaw cycle did not result in change in droplet size or phase separation or turbidity. All formulations showed good thermodynamic stability and were taken for storage stability.

Storage Stability

The microemulsions exhibited transparency and showed no evidence on phase separation or flocculation when they were subjected to stability study at 25°C±2°C and 60%±5% RH for 6 months. Average droplet size of prepared microemulsion batches were measured at different time intervals and the obtained results are depicted in Fig. 6. No significant difference was observed in the PDI of microemulsions up to 6 months (p>0.05).

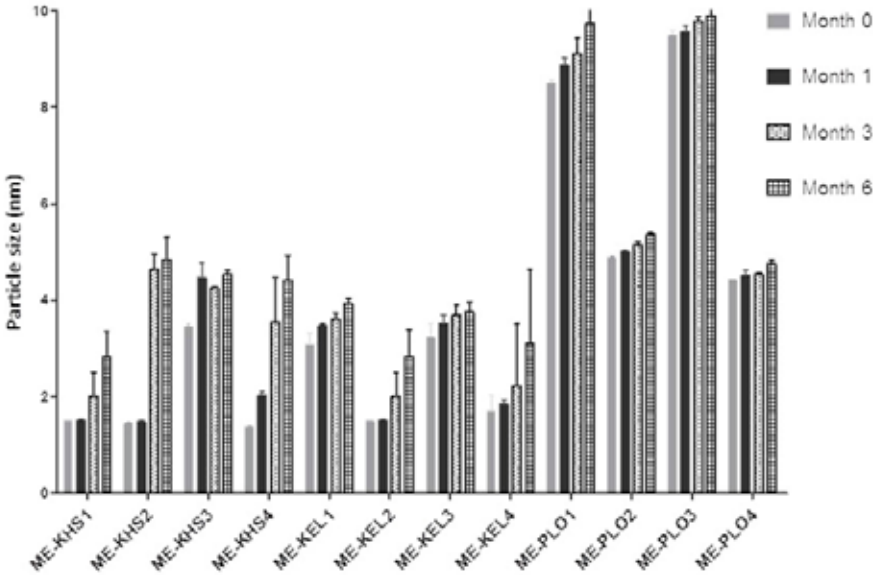

Figure 6: Droplet size and distribution of microemulsions after storage at 25±2°C and 60±5% RH for 6 months.

CONCLUSION

Microemulsion type colloidal carriers are one of the promising systems in skin penetration enhancement when compared with conventional formulations. Our results confirmed that the physicochemical characteristics of microemulsions are closely related to the type and ratio of the constituents and, the developed microemulsion formulations could be an alternative topical carrier to the current topical isotretinoin formulation available in the treatment of mild acne.

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UMCA SOLUSYON BİLEŞİMİ: Her 100 g çözelti etkin madde olarak 80 g Pelargonium sidoides kökü sıvı ekstresi, çözücü ve koruyucu olarak etanol ve gliserol içermektedir. TIBBİ ÖZELLİKLERİ: Umca Pelargonium sidoides'in kökünden elde edilen bir öz içermektedir. Pelargonium sidoides'ten elde edilen özün bronşit, sinüzit, anjin (boğaz ağrısı), viral enfeksiyonlara bağlı burun akıntısı ve farenjit olgularında etkili olduğu saptanmıştır. Aynı zamanda bağışıklık sistemini güçlendirici antiviral özelliklere sahiptir. Ayrıca bazı bakterilere karşı antibakteriyel etkisinin yanısıra antioksidan özelliklerine sahiptir. Bunun dışında, organizmanın bağışıklık sistemini güçlendirdiği ve solunum yolu mukozasındaki titrek tüylerin vurum sıklığını arttırarak balgam söktürücü etkiye sahip olduğu da bildirilmiştir. Bu nedenle Umca, değişik akut ve kronik enfeksiyonların özellikle de üst solunum yolları enfeksiyonları ve kulak-burun-boğaz enfeksiyonlarının tedavisi için uygundur. Umca uygulaması ile öksürük, ateş, boğaz ağrısı, halsizlik-yorgunluk gibi yakınmalarda hızlı bir iyileşme sağlanabilmektedir. ÖNERİLEN KULLANIM YERİ: Umca, akut ve kronik enfeksiyonlar, özellikle de solunum yolları enfeksiyonları (örneğin soğuk algınlığı ve bronşit gibi) ve kulak-burun-boğaz enfeksiyonları (örneğin sinüzit, anjin, rinofarenjit gibi) tedavisine yardımcıdır. Umca öksürük, ateş, boğaz ağrısı, halsizlik-yorgunluk gibi yakınmaların tedavisine yardımcıdır. KULLANILMAMASI GEREKEN DURUMLAR: Umca'nın içinde bulunan maddelerden herhangi birine karşı aşırı hassasiyeti olanlarda kullanılmamalıdır. Kanama eğiliminin yüksek olduğu hastalarda veya kan pıhtılaşması önleyen ilaçlar alanlarda kullanılmamalıdır. Yeterli klinik deneyim olmadığı için, ağır karaciğer ve böbrek rahatsızlıkları olanlarda kullanılmamalıdır. UYARILAR/ÖNLEMLER: Umca 1 yaşın altındaki bebeklerde önerilmemektedir. Gebeler veya emziren anneler tarafından kullanımı önerilmemektedir. Etanol içermesi nedeniyle araç ve makine kullanımında dikkatli olunmalıdır. YAN ETKİLER/ADVERS ETKİLER: Enfeksiyon durumlarında örneğin akut bronşitte karın ağrısı, mide yanması, bulantı ve ishal gibi yakınmalar görülebilir. Nadiren, bu yakınmalar Umca kullanımına bağlı olabilir. Nadir vakalarda, hafif dış eti veya burun kanaması görülebilir. Umca'nın içinde bulunan maddelere karşı aşırı hassasiyeti olanlarda çok nadiren aşırı duyarlılık reaksiyonlar gelişebilir. Bu tür reaksiyonlarda yüzde ödem (şişlik), nefes darlığı ve kan basıncında düşüş görülebilir ve ürünün ilk alımından sonra gelişebilir. Böyle bir durumda derhal doktora başvurulmalıdır. İstenmeyen bir etki görüldüğü zaman Sağlık Bakanlığı Türkiye Farmakovijilans Merkezi (TÜFAM)'ne bildiriniz. İLAÇ ETKİLEŞİMLERİ VE DİĞER ETKİLEŞİMLER: Kumarin türevleri ile birlikte kullanılması durumunda, kan pıhtılaşmasını engelleyici etkide bir artış meydana gelebilir. Bu nedenle koagülasyonu inhibe eden ilaçlar ile birlikte kullanılmamalıdır. GÜNLÜK KULLANIM ŞEKLİ VE DOZU: Yetişkinler ve 12 yaş üzeri çocuklarda günde 3 defa 30 damla, 6-12 yaş arası çocuklarda günde 3 defa 20 damla, ve 1-5 yaş arası çocuklarda günde 3 defa 10 damla şeklinde kullanılır. Damlalar, yemeklerden 30 dakika önce bir miktar sıvı ile birlikte içilmelidir. Hastalığın nüksetmemesi için, hastalığın belirtileri hafiflemesini takiben ilacın kullanımına birkaç gün daha devam edilmesi önerilir. Umca şişesi açıldıktan sonra oda ısında muhafaza edildiği takdirde 6 ay boyunca kullanılabilir. TİCARİ TAKDİM ŞEKLİ: 20 ve 50 ml'lik kendinden damlalıklı cam şişelerde. İZİN SAHİBİ: Abdi İbrahim İlaç San. ve Tic. A.Ş. Reşitpaşa Mah. Eski Büyükdere Cad. No:4 34467 Maslak / Sarıyer / İSTANBUL İZİN TARİH VE NUMARASI: 11.03.2008-2008/10 ÜRETİM YERİ & LİSANS SAHİBİ: Dr. Willmar Schwabe GmbH & Co. KG Willmar-Schwabe Straße 4, 76227, Karlsruhe / ALMANYA PAREKENDE SATIŞ FİYATI: 20 mL solusyon 35 TL, 50 mL solusyon 59 TL "25°C'nin altındaki oda sıcaklığında saklayınız." "Çocukların göremeyeceği, erişemeyeceği yerlerde ve ambalajında saklayınız." BU ÜRÜNÜN TIBBİ YARARI GELENEKSEL KULLANIMA VE LİTERATÜRE DAYANMAKTADIR. TIBBİ MÜSTAHZAR (İLAÇ) OLARAK DEĞERLENDİRİLMEMİŞTİR. SADECE ECZANELERDE SATILIR.

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Formulation And Characterization Of Intra Nasal Delivery Of Nortriptyline Hydrochloride Thermoreversible Gelling System In Treatment Of Depression

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ABSTRACT

The purpose of the present study is to develop a thermoreversible intra nasal gel of Nortriptyline hydrochloride (NTH). The prepared formulations were evaluated for gelation temperature, viscosity, gel strength, mucoadhesion strength, and drug content, *ex vivo* drug permeation, and stability study. The results found that as the concentration of poloxamer 188 and HPMC K4M were increased, there was increase in viscosity and mucoadhesive strength and decrease in gelation temperature and percent drug permeation. The optimized formulation F4 containing 3.6% poloxamer and 0.04% HPMC K4M showed highest drug release 98.25 % through sheep nasal mucosa. The intra nasal gel was stable for 3 months. It was concluded that, developed thermoreversible intra nasal formulations increased patient complain in treatment of a depression.

Keywords: Intra nasal gel, Nortriptyline hydrochloride, poloxamer

INTRODUCTION

Depression is a common psychiatric disorder affecting about 120 million people worldwide, and statistics clearly identify it as a major public health problem.[1]. Conventional oral dosage forms are available in market for treatment of depression, but the major drawbacks with these are many patients find it difficult to swallow (dysphagia) tablets and capsules. The difficulty experienced mainly in pediatrics and geriatrics patients[2]. The main advantages of nose to brain delivery such as rapidly absorbed through the nasal mucosa, preventing

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first pass metabolism and dodging of BBB. These nerve pathways initiate in the nasal cavity at olfactory neuroepithelium and terminate in the brain [3-4]. Both hydrophobic drugs, e.g. propranolol [5] and hydrophilic drugs, e.g. vanlafaxine hydrochloride [6] are absorbed by the nasal mucosa. Nortriptyline hydrochloride (NTH) is a tricyclic antidepressant widely used in the treatment of unipolar depression[7]. Furthermore, it has been reported that up to 70% of patients who are prescribed oral antidepressants fail to take 25–50% of their prescribed dose[8]. Low oral bioavailability (30-50%) and plasma level fluctuations such problems may cause lack of patient compliance and possible failure of the therapy [9-8]. Biodegradable, thermosensitive polymers have been extensively studied for their utility in formulation of thermoresponsive intranasal hydrogels [10-11-12]. In order to formulate thermosensitive intra nasal gel, thermoreversible polymer must have gelation temperature in the nasal physiological temperature range (29°C to 34°C). Poloxamers are nonionic, poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) copolymers (PEO–PPO–PEO), which form micelles at low concentrations and clear thermoreversible gels at high concentrations [13]. Using hydrophilic excipients, gelation temperature of poloxamer blends can be modulated so that they form in-situ gels at body temperature[14]. Numbers of literature have reported the use of poloxamer gels in intranasal administration of drugs like anti-emetics [15], anti-migraine agents[16]. Poloxamer 188 has low toxicity and biocompatibility, easy gel preparation methods, good compatibility with drugs and pharmaceutical excipients [17-23]. The objective of the present work was to develop a nose to brain delivery of Nortriptyline hydrochloride to enhanced patient compliance in treatment of depression.

METHODOLOGY

Materials

Nortriptyline Hydrochloride (NTH) was a gift sample from Swapnaroop drugs and chemicals, Aurangabad, India. Poloxamer-188 was procured as gift samples from BASF India Limited, Mumbai. HPMC K4M was a gift sample from Colorcon, Mumbai, India. Sodium metabisulpite and Benzalkonium chloride were obtained from Loba Chemie Pvt Ltd, Mumbai. DMSO was purchased from Vijay scientific Pvt Ltd. All other chemicals were of analytical reagent grade.

Methods

Preparation of intra nasal gel

The gel was prepared using the cold method [24]. The poloxamer-188 was slowly added and dissolved in cold water (5°C) with continues stirring by mechanical stirrer (Remi motors Ltd, Mumbai, India, type RQ-122). The dispersion were

then stored in refrigerator until clear solution was obtained. HPMC K4M with different concentration (0.04, 0.08 and 0.12%) was dissolved in ultra-pure water and stirred for 60 min. From the each prepared HPMC K4M solution NTH (0.2% w/v) was added which was previously solubilized in co-solvent DMSO. Poloxamer 188 solution was added in HPMC solution containing drug with continuous stirring. Appropriate amount of benzalkonium chloride, sodium metabisulphite were added simultaneously. The prepared formulations are shown in Table 1.

Table 1: Formulations of Nortriptyline HCl

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Nortriptyline HCl (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Ploxamer 188 (%)	3.4	3.4	3.4	3.6	3.6	3.6	3.8	3.8	3.8
HPMC K4M (%)	0.04	0.08	0.12	0.04	0.08	0.12	0.04	0.08	0.12
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Sodium metabisulphite	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Ultra-pure water (q.s) ml	100	100	100	100	100	100	100	100	100

Determination of gelation temperature

Two milliliter aliquot of gel was transferred to a test tube, immersed in a water bath. The temperature of water bath was increased slowly at a constant rate of 1°C for 2 min from room temperature to the temperature at which gel formed. The sample was then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting the test tube through an angle of 90° [25].

Viscosity study

The viscosity of *intra nasal* gel formulation before and after gelation was determined using Brookfield Rheometer R/S-CPS +1600 (Lauda Ecoline Staredition RE-204)[26] having cone and plate geometry by using spindle coaxial CP75-1.

Gel strength study

Test was performed using a gel strength apparatus modified at the laboratory. Intra nasal gel formulation (50g) was placed in a 100 ml-measuring cylinder and gelation was induced by simulated nasal fluid. The apparatus for measuring gel strength (weight: 35 g) was then placed on the gel. The gel strength was measured by the minimal weight that pushed the apparatus 5 cm down through the gel[27].

Mucoadhesive strength study

The fresh nasal mucosa was carefully removed from nasal cavity of sheep obtain from local slaughter house (Aurangabad). The nasal mucosa was mounted

on glass surface using adhesive tape while another mucosal section was fixed in inverted position to the cylinder. 50 mg of gel was placed on mucosal surface. The glass mounted mucosal surface with gel formulation and mucosal surface attached to cylinder were held in contact with each other for 2 min to ensure intimate contact between them. In second pan, the weights were increased until the two mucosal tissues got detached from each other. The nasal mucosa was changed for each measurement. The mucoadhesive force expressed as the detachment stress in dynes/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation[28].

Mucoadhesive strength (dynes/cm^2) = mg/A

Where,

m = Weight required for detachment in gram,

g = Acceleration due to gravity (980cm/s^2),

A = Area of mucosa exposed.

Drug content

The appropriate amount of formulation was taken and diluted with phosphate buffer pH6.6 and filter. The drug content was determined by using UV-visible spectrophotometer (Shimadzu, UV-1800, Lab India) at λ_{max} 239 nm.

***Ex-vivo* permeation study**

Institutional animal ethical committee approved the protocol (1211/PO/Re/S08/CPCSEA). *Ex-vivo* permeation study of all formulations was carried out using Franz diffusion cell. Nasal mucosa was placed in diffusion cells displaying a permeation area of 0.785 cm^2. The receiver compartment containing phosphate buffer pH 6.6 was maintained at 37 ±0.5°C. After a pre-incubation time of 20 min, formulation equivalent to 20 mg of NTH was placed in the donor chamber. At predetermined time points (30, 60, 120, 180 and 240 min), 1ml of sample was withdrawn from the receptor compartment, replacing with fresh phosphate buffer pH 6.6 The samples withdrawn were filtered and amount of drug permeated was determined using UV-visible spectrophotometer (Shimadzu, UV-1800, Lab India) at λ_{max} 239 nm[29].

Release mechanism

The Ex vivo permeation data were fitted to zero order, first order, Higuchi release model, Hixson and Crowell method and Korsemeyer-Peppas model by using DD Solver software, and the model with the higher correlation coefficient was considered to be the best model.

The Korsemeyer-Peppas equation as follows:

$M_t/M = Ktn$

Log Mt/M = log K + n log t

Where, M_t/M=fraction of drug released at time t, K=release rate constant, n=is the diffusion exponent indicating the release mechanism.

When n is equal to 0.5, the drug release is with a fickian-diffusion mechanism (Higuchi model). If 0.5 < n > 1 this indicates anomalous or non-fickian release, while if n = 1 this indicates zero order release[30].

Stability study

The stability study was done on optimized formulation as per ICH guideline, at 40° C ± 2°C and humidity 75% RH ± 5% condition in stability chamber (HMG, India) for three months. The formulation was examined for pH, drug content and viscosity. [31]

RESULTS AND DISCUSSION

Gelation temperature study

All the formulation showed gelation temperature between 32± 1.0°C to 34± 1.0°C. From results, it revealed that concentration of poloxamer 188 increased, the gelation temperature decreased shown in table 2. This can be explained by one of the proposed mechanism of gelation of aqueous poloxamer solutions, packaging of micelles and micelle entanglements have been suggested. With increasing temperature, micellization becomes more important and then at a definite point micelles come into contact and no longer move [10] therefore, if the poloxamer concentration in an aqueous solution is higher, packaging of micelles will occur at lower temperatures. As the concentration of poloxamer 188 increases, the gel structure becomes more closely packed with the arrangement in the lattice pattern and gelling occurs rapidly at low temperature. From above results obtained, gelation temperature study of all formulations, it was seen that all formulation showed gel formation at nasal physiological temperature i.e 32°C to 35°C as reported [32].

Viscosity study

Viscosity of all formulation before and after gel formation shown in Table 2. From results it found that the concentration of HPMC increased (0.04-0.12%), the viscosity increased. Similarly the concentration of poloxamer increased (3.4-3.8%), the viscosity increased. This effect could be attributed to the increase in the number and size of the micelles formed at the higher polymer concentration.

Furthermore, higher polymer concentration could result in a shorter intermicellar distance, leading to greater number of cross-links between neighboring micelles and a greater number of micelles per unit volume [33]. The viscosity of both, solution and gel formulations, was found to be proportionate to the increase in polymer concentration.

Gel strength

The gel strength values between 25-50 sec are considered adequate. The gel strength less than 25 sec may not retain its integrity and may erode rapidly while gel having strength greater than 50 sec is too stiff and may cause discomfort to the mucosal surfaces. Formulations (F1-F9) had gel strength between 28.2 sec to 51.83 sec in triplicate as shown in Table 2 and were considered suitable for nasal administration.

Mucoadhesive strength study

Our study indicates that, the concentration of HPMC increases the mucoadhesive strength goes on increasing shown in Table 2. HPMC is a hydrophilic polymer with many polar functional groups. Upon hydration the polymeric chains of HPMC get entangled with glycoprotein chains of mucin resulting in bioadhesion [34-35]. The significant effect was observed with HPMC as compared to poloxamer. This was due to wetting and swelling of HPMC, which permits intimate contact with nasal tissue, interpenetration of mucoadhesive HPMC chains with mucin molecules leading to entanglement and formation of weak chemical bonds between entangled chains. Due to stronger mucoadhesive force, it can prevent the gelled solution coming out of the nose and increases its residence time in nasal cavity. The degree of bioadhesion depends on type and amount of polymer, excipients used in the dosage form, degree of hydration, polymer chain length and molecular weight of polymer [36-37].

Drug content

The percent drug content of all formulations was found to be in the range, 98.35-101.71% shown in table 2, which revealed that drug is uniformly distributed within formulation.

***Ex vivo* permeation studies**

Ex vivo permeation profile of all formulations was studied using sheep nasal mucosa. The results show that as the level of poloxamer was increased (3.4 to 3.8%), the drug release was decreased. These results indicated that the concentration of polymer increased, structure of the gel became more closely packed and functioned as an increasingly resistant barrier to drug release. The percent-

age drug permeated after 240 min of all formulations was found to be between 75.50 to 98.25% (Figure 1). As compared to all formulation F4 (98.25%) showed highest drug release and considered as optimized formulation for further stability study. The initial rates of permeation were very rapid due to incomplete gel formation, but as the time progresses the permeation rate decreases due to complete gel formation. With an increase in concentration of HPMC (0.04 to 0.12 %), the diffusion rates were found to decrease gradually. An increase in polymer concentration increases the viscosity of gel layer with longer diffusional path length resulting in greater retardation of drug in gel. The permeation profiles exhibited an inflection point, which indicated the gel formation in the donor compartment of diffusion cell. During gel formation, a portion of drug might be loaded into the gel matrix, thus the cross-linking of polymer reduces the drug permeation rate.

Table 2: Gelation temperature, viscosity, gel strength and mucoadhesive strength and drug content

Formulation Code	Gelation Temperature (°C)	Viscosity (cp)		Gel Strength (Sec)	Mucoadhesive Strength (dynes/ cm²)	Drug Content (%)	% Drug Permeated
		Sol	Gel				
F1	34± 1.0	33.16±0.3	451.16±0.76	28 ± 2	1784.46±1.2	99.25±0.12	81.53±2.2
F2	34± 1.0	39.06±0.6	553.5 ±1.04	32.66 ± 2.08	1994.70±1.2	101.71±0.2	73.52± 4.6
F3	34± 1.0	47.80±0.3	677.4±0.6	36.5 ± 0.25	2195.54±2.1	98.35±0.9	70.02± 5.8
F4	33± 1.0	54.35±0.2	751.83±1.60	37.3 ± 0.51	2450.69±2.40	99.81±0.4	98.25±2.6
F5	33± 1.0	63.67±0.5	842.6 ±0.52	42.13± 0.41	2557.49±2.16	98.62±0.5	91.48±5.4
F6	33± 1.0	72.31±0.5	850±1	44.66 ± 0.70	2359.54±3.3	99.21±0.7	82.22±4.4
F7	32± 1.0	82.75±0.3	985.2±0.8	38.73 ± 0.25	2670.2 ±4.3	98.91±0.8	84.28±5.5
F8	32± 1.0	97.26±0.3	1004.5±2.23	46.6 ± 0.52	2746.05±5.7	99.25±0.3	81.22±3.8
F9	32± 1.0	107.60±0.8	1011.5±2.17	51.83± 0.73	2785.79±5.4	99.1±0.2	75.50±4.7

Release mechanism

The results obtained from release kinetics it could be concluded that, the formulations (F1 to F9) exhibited n values between 0.548 – 0.976 indicating an anomalous or nonfickian release suggesting a coupled erosion– diffusion mechanism.

Stability study

In stability study, there was no significant change found in drug content, pH and viscosity as shown in Table.3. From above results, it was concluded that prepared formulation is stable.

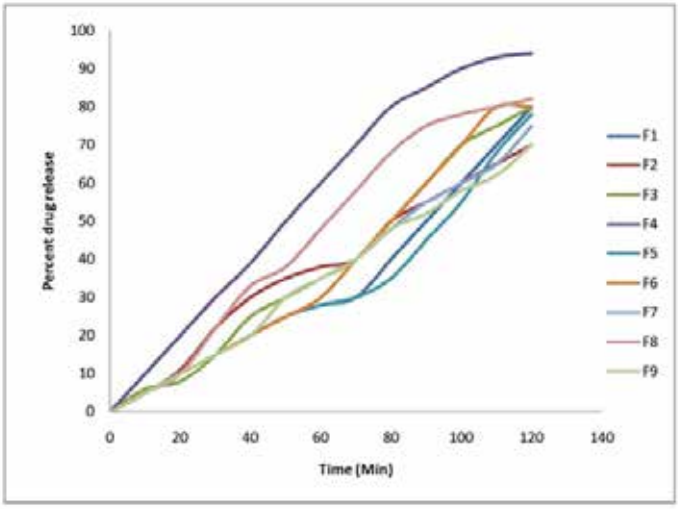

Figure 1: Ex vivo permeation profile of all formulations through sheep nasal mucosa

Table 3: Stability results of optimized F4 formulation

Days	Drug content (%)	pH	Viscosity (cp)
0	99.81±0.4	5.65±0.02	54.35±0.24
30	99.34±0.11	5.66±0.06	56.12±0.09
60	99.03±0.06	5.68±0.09	57.39±0.17
90	98.95±0.13	5.78±0.15	58.60±0.11

CONCLUSION

The optimized formulation F4 containing 3.6 % poloxamer 188 and 0.04 % HPMC K4M show highest drug release 98.25 % through sheep nasal mucosa. It was concluded that, the intra nasal formulations of Nortriptyline hydrochloride (NTH) was effectively formulated to enhanced patients compliance for the treatment of depression.

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Antimicrobial Activity Evaluation Of New 1,3,4-oxadiazole Derivatives

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ABSTRACT

In this study, we have synthesized seven novel 2-[(5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio]-*N*-(6-substituted benzothiazol-2-yl)acetamide derivatives **(4a-g)** starting from ethyl 4-chlorophenyl acetate. The antimicrobial acitivity of the compounds was screened against seven Gram positive and Gram negative bacteria and four fungi species; *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Enterococcus faecalis* ATCC 51299, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans, Candida krusei, Candida glabrata, Candida parapsilosis*. Minumum inhibitor concentration (MIC) was calculated and compared with standard drugs, chloramphenicol and ketoconazole. Regarding the results of MIC, all compounds exhibited potency either at the higher concentrations or at the same concentrations compared with positive controls.

Keywords: 1,3,4-oxadiazole, benzothiazole, antibacterial, antifungal

INTRODUCTION

Infectious diseases are one of the most deadly diseases in the world[1]. Recently, the number of bacterial and fungal infections has rised dangereously[2]. Antibiotics and antifungals are the most important drug groups used in the treatment of bacterial and fungal infections. With the discovery of antibiotics, these drugs have begun to be used as main drugs in the treatment of infections. But over time, bacteria have begun to develop resistance because of frequent use and misuse. An uncontrolled increase in resistance of pathogenic microorganisms has wasted health resources[3-5]. This resistance to antimicrobial agents has shown

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that there is an urgent need for new treatment strategies and new antimicrobial drug discovery studies.

Heterocyclic chemistry was discovered in the early 1800s. Heterocyclic members have an important area in organic chemistry because they have broad range of pharmacological effects[6]. Among them, oxadiazole is one of the prominent aromatic ring containing oxygen and nitrogen atoms. Due to its electronic and charge-transport properties it can be easily connected to various functional groups[7]. In addition, oxadiazoles have been extensively studied over recent years due to their different biological activities. This five member heterocyclic ring plays an important role in medicinal chemistrywhich exists in new molecules as pharmacophore groups[8,9]. Different classes of oxadiazoles have broad range of pharmacological activities such as antimalarial, anticonvulsant, analgesic, antimicrobial, antimycobacterial, antitumor, vasodilator, cytotoxic, hypolipidemic, antiproliferative, antifungal[10-17]. Some of prescribed agents possessing oxadiazole ring are antimicrobial furamizole[6], antiretroviral agent raltegravir and antihypertensive agent nesapidil[18] **(Figure 1)**.

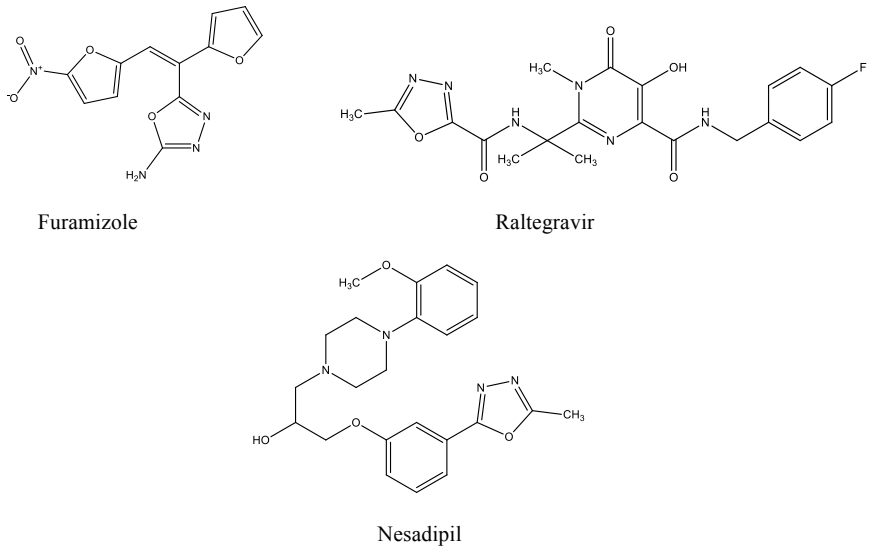


Figure 1. Some oxadiazole possessing drugs

Additionally, 1,3,4-oxadiazoles are good bioisosteres of amide and ester groups that exhibit different biological activities by making strong hydrogen bonds with different receptors[10,11]. On the other hand, 1,3,4-oxadizoles can react with the nucleophilic centers of microbial cells by reacting with the presence of the -N = C-O toxophoric group[18].

There are several methods in the literature for the synthesis of 1,3,4-oxadiazoles.

By using acid hydrazides, phosphorus oxychloride, sulfuric acid, and thionyl chloride, the oxadiazole ring was obtained in several steps[14,15,19]. However, the method of synthesis of 1,3,4-oxadiazoles by reaction of carboxylic acid and acid hydrazides is not a highly preferred method because it is expensive and requires a long time[20].

In this study, we have synthesized seven novel compounds combining 5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-thiol and 2-chloro-*N*-(2-benzothiazolyl) acetamide derivatives. The antimicrobial activity of the synthesized compounds was investigated against different microorganisms compared with standard drugs chloramphenicol and ketoconazole.

METHODOLOGY

Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO, USA) and Merck Chemicals (Merck KGaA, Darmstadt, Germany). All melting points (m.p.) were determined by MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were uncorrected. All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany). Spectroscopic data were recorded with the following instruments: IR, Shimadzu Affinity 1S spectrophotometer (Shimadzu, Tokyo, Japan); NMR, Agilent 300 MHz NMR spectrometer (Agilent technologies, California, USA), in DMSO-d_6, using TMS as internal standard; M+1 peaks were determined by Shimadzu 8040 LC/MS/MS system (Shimadzu, Tokyo, Japan). Elemental analyses were performed on a Leco 932 CHNS analyzer (Leco, Michigan, USA).

Synthesis of ethyl 4-chlorophenyl acetate (1)

4-Chlorophenyl acetic acid (0.40 mol) was refluxed with excess ethanol for 12h catalyzed with H_2SO_4. After TLC check, the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate, washed with salty water and dried with sodium sulfate. Ethyl acetate was evaporated under reduced pressure to gain ester compound (**1**).

Synthesis of 2-(4-chlorophenyl)acetohydrazide (2)

Ethyl 4-chlorophenyl acetate (0.25 mol) was dissolved in ethanol (150 ml). Hydrazine hydrate (0.50 mol) added and the mixture stirred in room temperature for 2h. After completion of reaction, the solvent was separated by filtration to acquire hydrazide compound **2.**

Synthesis of 5-(4-chlorobenzyl)-1,3,4-oxadiazole-2-thiol (3)

2-(4-Chlorophenyl)acetohydrazide (0.20 mol) was dissolved in ethanol (250 ml). 0. 24 mol of potassium hydroxide was dissolved in ethanol (100 mL)and added to the mixture. Secondly, carbon disulfide (0.60 mol) was added to the mixture, and it was refluxed for 5 hours. After this period, cold water and dilute HCl were added to the reaction mixture to gain product **3**.

Synthesis of 2-[(5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio]-N-(6-substituted benzothiazol-2-yl)acetamidederivatives (4a-g)

5-(4-Chlorobenzyl)-1,3,4-oxadiazole-2-thiol (10 mmol) was dissolved in acetone (50 mL), potassium carbonate (12 mmol) and appropriate 2-chloro-*N*-(2-benzothiazolyl)acetamide derivatives were added to this solution and stirred for 12h in room temperature. After TLC screening, the solvent was evaporated under reduced pressure then water was added to wash the resulting solid and the mixture was filtered, dried and recrystallized from ethanol to give final compounds **4a-g.**

2-[(5-(4-Chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio]-N-(benzothiazol-2-yl)acetamide (4a)

Yield: 69 %. M.p. 231°C.

^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.27 (2H, s, -CH_2), 4.40 (2H, s, -$COCH_2$), 7.30-7.37 (5H, m, Ar-H), 7.46 (1H, t, *J*= 7.44 Hz, Ar-H), 7.78 (1H, d, *J*= 7.95 Hz, aromatic-H), 7.99 (1H, d, *J*= 7.41 Hz, aromatic-H), 12.74 (1H, s, -NH).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 30.46 (CH_2), 36.01 (CH_2), 121.19 (CH), 122.26 (CH), 124.23(CH), 126.70 (CH), 129.10 (CH), 131.93 (CH), 132.48 (C), 133.59 (C), 148.98 (C), 158.06 (C), 163.43 (C), 166.75 (C), 166.97 (C).

For $C_{18}H_{13}ClN_4O_2S_2$ calculated: 51.86 % C, 3.14 % H, 13.44 % N, found: 51.82 % C, 3.15 % H, 13.47 % N.

HRMS (m/z): $[M+H]^+$calcd for $C_{18}H_{13}ClN_4O_2S_2$: 417.0241; found 417.0232.

2-[(5-(4-Chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio]-N-(6-methylbenzothiazol-2-yl)acetamide(4b)

Yield: 72 %. M.p. 244°C.

^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 2.41 (3H, s, -CH_3), 4.27 (2H, s, -CH_2), 4.39 (2H, s, -$COCH_2$), 7.25-7.38 (5H, m, Ar-H), 7.66 (1H, d, *J*= 8.22 Hz, Ar-H), 7.78 (1H, s, aromatic-H), 12.67 (1H, s, -NH).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 21.46 (CH_3), 30.46 (CH_2), 35.99 (CH_2), 121.83 (CH), 122.41 (CH), 123.35 (CH), 128.62 (CH), 129.10 (CH), 131.26 (C),

132.06 (C), 132.48 (C), 133.72 (C), 146.91 (C), 157.19 (C), 163.44 (C), 166.59 (C), 166.96 (C).

For $C_{19}H_{15}ClN_4O_2S_2$ calculated: 52.96 % C, 3.51 % H, 13.00 % N, found: 52.91 % C, 3.52 % H, 13.04 % N.

HRMS (m/z): $[M+H]^+$calcd for $C_{19}H_{15}ClN_4O_2S_2$: 431.0394; found 431.0394.

2-[(5-(4-Chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio]-N-(6-methoxybenzothiazol-2-yl)acetamide (4c)

Yield: 75 %. M.p. 241°C.

^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 3.80 (3H, s, -OCH_3), 4.26 (2H, s, -CH_2), 4.38 (2H, s, -$COCH_2$), 7.04 (1H, dd, *J*= 8.42 Hz, *J*= 2.58 Hz, Ar-H), 7.29-7.37 (4H, m, Ar-H), 7.57 (1H, s, aromatic-H), 7. 65 (1H, d, *J*= 8.85 Hz, 12.61 (1H, s, -NH).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 30.46 (CH_2), 35.96 (CH_2), 56.10 (OCH_3), 121.79 (CH), 124.45 (CH), 128.62 (CH), 129.09 (CH), 131.26 (CH), 132.48 (C), 133.25 (C), 133.59 (C), 143.00 (C), 156.00 (C), 156.74 (C), 163.45 (C), 166.44 (C), 166.95 (C).

For $C_{19}H_{15}ClN_4O_3S_2$ calculated: 51.06 % C, 3.38 % H, 12.54 % N, found: 51.11 % C, 3.39 % H, 12.58 % N.

HRMS (m/z): $[M+H]^+$calcd for $C_{19}H_{15}ClN_4O_3S_2$: 447.0347; found 447.0328.

2-[(5-(4-Chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio]-N-(6-ethoxybenzothiazol-2-yl)acetamide (4d)

Yield: 68 %. M.p. 233°C.

^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 1.35 (3H, t, *J*= 6.93 Hz, -CH_3), 4.07 (2H, q, *J*= 6.90 Hz, -OCH_2), 4.27 (2H, s, -CH_2), 4.37 (2H, s, -$COCH_2$), 7.03 (1H, dd, *J*= 8.70 Hz, *J*= 2.49 Hz, Ar-H), 7.30-7.37 (4H, m, Ar-H), 7.56 (1H, s, aromatic-H), 7. 65 (1H, d, *J*= 8.82 Hz, 12.59 (1H, s, -NH).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 15.15 (CH_3), 30.46 (CH_2), 35.95 (CH_2), 64.08 (OCH_2), 121.80 (CH), 124.80 (CH), 129.10 (CH), 131.26 (CH), 132.47 (C), 133.25 (C), 133.60 (C), 135.12 (C), 142.96 (C), 155.96 (C), 163.45 (C), 166.39 (C), 166.96 (C).

For $C_{20}H_{17}ClN_4O_3S_2$ calculated: 52.11 % C, 3.72 % H, 12.15 % N, found: 52.15 % C, 3.73 % H, 12.18 % N.

HRMS (m/z): $[M+H]^+$calcd for $C_{20}H_{17}ClN_4O_3S_2$: 461.0503; found 461.0494.

2-[(5-(4-Chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio]-N-(6-chlorobenzothiazol-2-yl)acetamide (4e)

Yield: 71 %. M.p. 247°C.

^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.28 (2H, s, CH_2), 4.41 (2H, s, -CH_2CO), 7.31-7.37 (4H, m, Ar-H), 7.47 (1H, dd, *J*= 8.70 Hz, *J*= 2.22 Hz, Ar-H), 7.77 (1H, d, *J*= 8.64 Hz, aromatic-H), 8.14 (1H, d, *J*= 2.13 Hz, Ar-H),12.85 (1H, s, -NH).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 30.46 (CH_2), 35.97 (CH_2), 121.98 (CH), 122.39 (CH), 127.05 (CH), 128.29 (C), 128.63 (C), 129.08 (CH), 131.24 (CH), 132.48 (C), 133.57 (C), 147.84 (C), 158.95 (C), 163.40 (C), 166.97 (C).

For $C_{18}H_{12}Cl_2N_4O_2S_2$ calculated: 47.90 % C, 2.68 % H, 12.41 % N, found: 47.81 % C, 2.69 % H, 12.45 % N.

HRMS (m/z): $[M+H]^+$calcd for $C_{18}H_{12}Cl_2N_4O_2S_2$: 450.9851; found 450.9832.

2-[(5-(4-Chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio]-N-(6-florobenzothiazol-2-yl)acetamide (4f)

Yield: 69 %. M.p. 241°C.

^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.26 (2H, s, CH_2), 4.39 (2H, s, -CH_2CO), 7.27-7.36 (5H, m, Ar-H), 7.76-7.81 (1H, m, Ar-H), 7.91(1H, dd, *J*= 8.70 Hz, *J*= 2.64 Hz, aromatic-H), 12.76 (1H, s, -NH).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 30.46 (CH_2), 35.94 (CH_2), 108.55 (C), 108.90 (C), 114.68 (C), 115.00 (CH), 122.27 (CH), 122.40 (CH), 129.09 (CH), 131.26 (CH), 132.47 (C), 133.59 (C), 157.62 (C), 160.80 (C), 163.41 (C), 166.85 (C), 166.97 (C).

For $C_{18}H_{12}ClFN_4O_2S_2$ calculated: 49.71 % C, 2.78 % H, 12.88 % N, found: 49.81 % C, 2.77 % H, 12.84 % N.

HRMS (m/z): $[M+H]^+$calcd for $C_{18}H_{12}ClFN_4O_2S_2$: 435.0147; found 435.0137.

2-[(5-(4-Chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio]-N-(6-nitrobenzothiazol-2-yl)acetamide (4g)

Yield: 73 %. M.p. 245°C.

^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.26 (2H, s, CH_2), 4.43 (2H, s, -CH_2CO), 7.29-7.37 (4H, m, Ar-H), 7.92 (1H, d, *J*= 8.97 Hz, Ar-H), 8.28 (1H, dd, *J*= 8.70 Hz, *J*= 2.43 Hz, aromatic-H), 13.15 (1H, s, -NH).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 30.46 (CH_2), 36.03 (CH_2), 119.61 (CH), 121.30 (CH), 122.30 (CH), 129.08 (CH), 131.24 (CH), 132.47 (C), 132.68 (C), 133.57 (C), 143.60 (C), 153.83 (C), 163.35 (C), 163.60 (C), 167.00 (C), 167.56 (C).

For $C_{18}H_{12}ClN_4O_4S_2$ calculated: 46.81 % C, 2.62 % H, 15.16 % N, found: 46.89 % C, 2.63 % H, 15.21 % N.

HRMS (m/z): $[M+H]^+$calcd for $C_{18}H_{12}ClN_4O_4S_2$: 462.0092; found 462.0084.

Antimicrobial activity

Antimicrobial activity against *Escherichia coli* (ATCC 25922), *Escherichia coli* (ATCC 35218), *Enterococcus faecalis* (ATCC 51299), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 22019), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 24433), *Candida krusei* (ATCC 6258), *Candida glabrata* (ATCC 90030), *Candida parapsilosis* (ATCC 90030) was determined by the microbroth dilutions technique using the Clinical Laboratory Standarts Institute (CLSI) recommendations[21].

The lowest concentration that completely inhibited growth of the microorganism was defined as the minimum inhibitor concentration (MIC). MIC was screened and the results were compared to chloramphenicol and ketoconazole as positive controls. Each experiment was replicated twice.

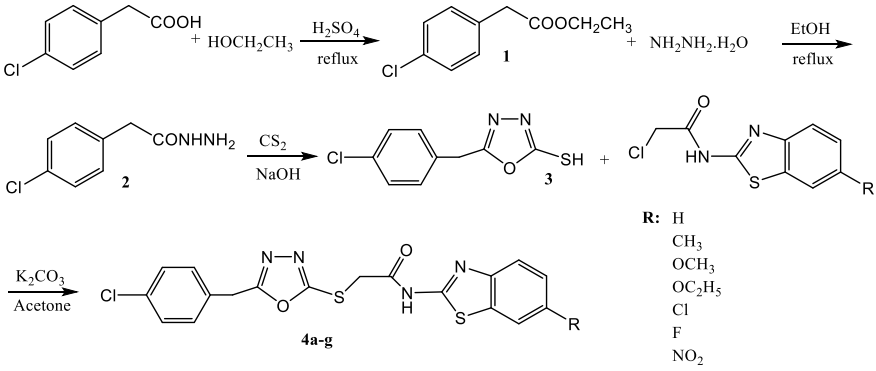
RESULTS AND DISCUSSION

Chemistry

In this study, we have synthesized 2-[(5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-yl) thio]-*N*-(6-substituted benzothiazol-2-yl)acetamide derivatives (**4a-g**) with four step synthetic procedure as shown **Scheme 1**. In the first step, compound **1** was synthesized by reacting 4-chlorophenyl acetic acid with H_2SO_4 in ethanol at reflux conditions. In the second step, compound **2** was synthesized by reacting 4-chlorophenyl acetate with hydrazine hydrate in ethanol at the room temperature. In the third step, compound **3** was synthesized by reacting 2-(4-chlorophenyl)acetohydrazide with potassium hydroxide and carbon disulfide in ethanol at the reflux conditions. In the last step, compounds **4a-g** were synthesized by reacting 5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-thiol with 2-chloro-*N*-(2-benzothiazolyl)acetamide derivatives in acetone at room temperature. The gained raw products were crystallized from ethanol.

In ^{1}H-NMR spectra of the synthesized compounds (**4a-g**), peaks of acetamide ($-CH_2CONH-$) moiety belongs to methylene and amide protons were identified at about 4.37-4.43 ppm and 12.59-13.15 ppm, respectively. If we look at the characteristic ^{1}H-NMR properties of the molecule, we detected peaks of methyl group ($-CH_3$) at 2.41 ppm for **4b,** peaks of methoxy group ($-OCH_3$) at 3.80 ppm for **4c**. For compound **4d,** peaks of methyl ($-CH_3$) and methylene group ($-OCH_2$) were

seen at 1.35 ppm and 4.07 ppm as broad singlet peaks. In the ^{13}C-NMR spectra, all carbons were determined between 15.15-167.56 ppm range. The peaks resonated at about 30.46 ppm and 35.94-36.03 ppm were assigned to $-SCH_2-$ and $-CCH_2-$ carbons, respectively. In the ^{13}C-NMR spectra of the compounds **4b, 4c** and **4d**, signals at 21.46, 56.10 and 15.15, 64.08 ppm were assigned to the carbon atoms of methyl, methoxy and ethoxy groups on benzothiazole ring. In aromatic region, signals with higher values were determined for the carbon atoms of the heterocyclic rings. In the MS spectra of the compounds, M^+ peaks were observed in agreement with molecular weights of the compounds. Elemental analysis for C, H, O atoms were within +/- 0.4 % of the theoretical values.



Scheme 1. The synthesis of the compounds (**4a-g**).

Biology

All synthesized compounds were tested for determining their antimicrobial activity against seven Gram positive and Gram negative bacterial and four fungal microorganisms; *E.coli* ATCC 25922, *E. coli* ATCC 35218, *E. faecalis* ATCC 51299, *E. faecalis* ATCC 29212, *S. aureus, K.pneumoniae, P. aeruginosa, C. albicans, C.krusei, C.glabrata, C. parapsilosis*. MIC values were determined against standard drugs, ketoconazole and chloramphenicol and represented in **Table 1.** MIC values of the compounds were found between 50-100 μg/ml and they were identified between 12.5-50 μg/ml for reference drugs. Compound **4a** showed antimicrobial activity against all microorganisms at 50μg/ml concentration. Compounds **4b, 4c, 4e** and **4g** exhibited activity against *E. coli* ATCC 35218 and *P. aeruginosa* at 100 μg/ml and against other bacteria at 50 μg/ml. MIC values were calculated against *P. aeruginosa* as 100 μg/ml for compound **4d** and against *E. coli* ATCC 35218 as 100 μg/ml for **4f**. Additionally, all compounds showed potency at the higher concentration against six bacteria *E.coli* ATCC 25922, *E. coli* ATCC 35218, *E. faecalis* ATCC 51299, *E. faecalis* ATCC 29212, *S. aureus, K.pneumoniae*. **4a** and **4f** exhibited same potency against *P.*

aeruginosa compared with chloramphenicol. All compounds showed lower activity against *C. albicans* than ketoconazole and they exhibited same potency against three fungal microorganisms *C. krusei, C. glabrata, C. parapsilosis* compared reference.

Table 1. Antimicrobial activities of the compounds (µg/mL)

Comp.	A	B	C	D	E	F	G	H	I	J	K
4a	50	50	50	50	50	50	50	50	50	50	50
4b	50	100	50	50	50	50	100	50	50	50	50
4c	50	100	50	50	50	50	100	50	50	50	50
4d	50	50	50	50	50	50	100	50	50	50	50
4e	50	100	50	50	50	50	100	50	50	50	50
4f	50	100	50	50	50	50	50	50	50	50	50
4g	50	100	50	50	50	50	100	50	50	50	50
Ref. 1	12.5	12.5	25	25	25	12.5	50	-	-	-	-
Ref. 2	-	-	-	-	-	-	-	25	50	50	50

Reference 1: Chloramphenicol, Reference 2: Ketoconazole

A: *E. coli* ATCC 25922, **B**: *E. coli* ATCC 35218, **C**: *E. faecalis* ATCC51299, **D**: *E. faecalis* ATCC 29212, **E**: *S. aureus*, **F***: K. pneumonia,* **G**: *P. aeruginosa,* **H**: *C. albicans,* **I:** *C. krusei,* **J:** *C. glabrata,* **K:** *C. parapsilosis.*

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Formulation Design of the Oral Disintegrating Tablets Including Alfuzosin Hydrochloride with Risk Evaluation via Quality by Design

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ABSTRACT

In this study, within the framework of Quality by Design which is a systematically scientific approach which enables to understand and control the production and formulation variables during the process design and development, different parameters in the formulation and production process were detected and critical process parameters and critical material attributes were determined via risk evaluation methods. Then, different oral disintegrating tablet formulations were prepared and tested by changing the usage of co-formulated disintegrating excipient and other disintegrant combined with sodium starch glycolate and mannitol. Powder flow characteristics were examined. Suitable formulations compressed via direct compression method at two different pressure levels. Compressed tablets were tested physically and chemically. The results thus obtained were evaluated in the Artificial Neural Network and Gene Expression Programming modules.

Keywords: Oral Disintegrating Tablets, Alfuzosin Hydrochloride, Quality by Design, Artificial Neural Network, Gene Expression Programming

INTRODUCTION

Today, it is generally accepted that quality cannot be tested or inspected into a finished product, but rather that quality, safety and effectiveness must be "designed" and built into a product and its manufacturing process. In the traditional approach, the production processes and process parameters are determined to be unchanged in order to avoid any variety in the quality of the product. Thus, required specifications are met and the product quality is measured by finished product tests[1]. In order to design quality into a product, the requirements for the product design and performance must be well understood in the early design phase. In pharmaceuticals, these product requirements can be justified as Quality Target Product Profile (QTPP) which is also defined as "a prospective summary of

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the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product"[2].

On the other hand, Quality by Design (QbD) method identifies characteristics that are critical to quality from the perspective of patients, translates them into attributes that the drug product should possess, and establishes how the process factors can be varied to consistently produce a drug product with the desired characteristics. The QbD approach begins with a predefined target product profile (TPP), and applies various principles and tools at different stages to better understand the product. Quality risk assessment (QRA) tools, such as Failure Mode Effects Analysis (FMEA) and Risk ranking and filtering, is applied to identify an initial list of potential Critical Quality Attributes (CQAs), Critical material attributes (CMAs) and Critical Process Parameters (CPPs) with risk assessment for each unit operation includes considering and documenting all parameters that could affect outputs (CQAs)[3-4].

CQAs mainly refer to quality attributes of raw material, intermediate or final product[5]. CQAs are those physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range or distribution to ensure the desired product quality. CQA assessment is an iterative process of evaluating the drug product and drug substance attributes throughout development to determine which have potential impact on the safety, efficacy, or potency of the drug[6].

The process and product design applied by QbD approach decrease the role of finished product tests and so ensure to be able to control quality at the design stage. When compared with traditional development approaches, formulation and manufacturing process dynamics are better understood in QbD approach. Moreover, the formulation contributes understanding the effect of production processes on product reliability and efficacy[7].

Design of Experiments (DoE) is a part of QbD and defined as a structured and organized method to determine the relationship among factors that influence outputs of a process. DoE results can help identify optimal conditions, the critical factors that most influence CQAs. Based on the acceptable range of CQAs, the design space of CPPs can be determined[8].

In ICH Q8[9], Design Space is defined as "the multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality"[8]. Working within this space is not considered as a change and hence does not require regulatory approval.

The latest advances in mathematics and computer science have developed methods that may aid complex data analysis throughout DoE, optimisation with mod-

elling and creating design space, and thus, different software products based on mathematical models have been developed to help to better understand the relationship between formulation and process parameters, ensures quality of product and to save time and money[10]. There are many computer software's employing Artificial Neural Network (ANN), Gene Expression Programming (GEP) and Neuro-Fuzzy Logic Modelling infrastructure for this purpose serving the pharmaceutical industry[11]. One of these software's is INForm of Intelligensys Ltd., UK that employs multilayer perceptron neural networks.

Artificial neural networks are calculation models inspired by biology that consist of hundreds of units and artificial neurons connected by factors (weights) that establish the neural network without any linear relationship. Nonetheless, the most important factor in deciding to use neural networks to solve a problem concerns whether the data represent the solution of the problem[10].

On the other hand, GEP is a transactional process producing best fitting integral solutions based on the principle of the most powerful survives (natural selection) in the complicated and multidimensional research fields and by mapping complex neural networks of different shapes and sizes (phenotype) in this process, they use linear chromosomes (genotype)[12].

Orally Disintegrating Tablet (ODT) is a solid unit dosage form, which disintegrates or dissolves rapidly in the mouth without chewing and water. This type of property in dosage form can be attained by addition of different excipients, from which disintegrating excipient is the key adjuvant[13]. ODTs are appreciated by a significant segment of populations particularly who have difficulty in swallowing. It has been reported that Dysphagia (difficulty in swallowing) is common among all age groups and more specific with paediatric, geriatric population along with psychiatric patients and patients with nausea, vomiting, and motion sickness complications[14].

Formulation properties and process parameters affect the disintegration time of ODTs. In this kind of tablets, direct compression is the most common used technique that requires the integration of disintegrants into the formulation to achieve the fast disintegration of tablets. To decrease the disintegration time of the tablet, it is necessary to avoid increasing the mechanical strength of ODTs. The mechanical strength of a tablet is related to its compression pressure and friability is inversely related to compression pressure. To ensure the quality of an ODT, these two properties should be properly balanced. ODTs can be soft, fragile so unsuitable for packaging in conventional blisters or bottles because of their low compression pressure, it is therefore necessary to develop a strategy to increase the tablet's mechanical strength without sacrificing its porosity or requiring special unit dose packaging, which may add to the cost of handling fragile tablets[15].

Alfuzosin hydrochloride is an alpha-adrenoreceptor blocker used in the manage-

ment of hypertension and it also relieves symptoms of urinary obstructions in benign prostatic hyperplasia[16]. The concept of formulating orally disintegrating tablets containing alfuzosin offers a suitable and practical approach in serving desired objective of rapid disintegration and dissolution characteristics with increased bioavailability[17]. In this study, alfuzosin hydrochloride was chosen as the model medication because of its low-dosage active ingredient and its indication.

In our study, different formulations were tested by changing the ready to use co-formulated disintegrant excipient (Ludiflash or combined sodium starch glycolate and mannitol), disintegrant % rate (30-80 %) and tablet compression pressure (500 or 1000 psi). Thereafter, the relationships between the formulation and process parameters and the target product properties (tablet hardness, friability, wetting time, water absorption ratio and disintegration time) and the pharmaceutically acceptable ODT formulation were determined using ANN and GEP models.

METHODOLOGY

Materials

Alfuzosin hydrochloride was obtained from Generica Drug Company (Turkey) as a gift. Avicel PH 101 (microcrystalline cellulose NF) was from FMC Biopolymer (Brussels, Belgium), and Ludiflash® was from BASF (Germany), Sodium stearyl fumarate was from SPI Pharma (U.S.A.), sodium starch glycolate was from DFE Pharma (Germany) and Mannitol was from Merck Co. (Germany). All other solvents and chemicals used were of analytical grade.

Methods

Creating Knowledge Space

First step of QbD framework starts with definition of CQA and application QbD by unit operation, working backwards from Drug Product after definition of QTPP. Subsequently, it continues with risk assessment on each unit operation and conduct designed experiments.

Quality Target Product Profile (QTPP)

The QTPP is derived from the desired labelling information that describes anticipated indications, contraindications, dosage form, dose, frequency, pharmacokinetics, and so on, for a new product[18]. There are various ways to represent a QTPP for ODT and one of them was given in Table 1.

Identify CQAs

CQAs are derived from QTPP and scientific rationale for CQAs should be explained. Table 2 summarizes the quality attributes of ODTs and indicates which attributes were classified as drug product CQAs.

Table 1: Quality Target Product Profile (QTPP) for ODT.

Quality Target Product Profile (QTPP) for ODT		
QTPP Element	**Target**	**Justification**
Dosage form	ODT	Patient compliance
Route of administration	Oral	Patient compliance
Dosage strength	2,5 mg	Maximum effect
	Physical Attributes (hardness, friability)	Pharmaceutical limit requirement
	Disintegration time	
	Wetting time	
	Water Content	
	Content Uniformity	
	Drug Release	
	Microbial Limits	

Table 2: Critical Quality Attributes (CQAs) of ODTs.

Critical Quality Attributes (CQAs) of ODTs			
Quality Attributes of the Drug Product	**Target**	**Is it a CQA?**	**Justification**
Appearance	Colour and shape acceptable to the patient. No visual tablet defects observed.	No	Colour, shape and appearance are not directly affect safety and efficacy. Therefore, they are not critical. . The target is set to ensure patient acceptability.
Size	< 20 mg (amount of active ingredient)	Yes	Size is critical as it affects wetting time and disintegration
Odour, taste	No unpleasant odour and taste	Yes	Odour and taste are critical in ODTs owing to patient convenience
Friability	< % 1	Yes	High friability causes decrease in size
Hardness	Pharmacopeia acceptability	Yes	Hardness affect disintegration time and drug efficiency
Disintegration time	< 3 minutes	Yes	Disintegration time affects efficiency
Wetting time	Minimum	Yes	Wetting time affect disintegration time
Water absorption capacity	Minimum	Yes	When the water absorption capacity is high more saliva is required to disintegrating ODT in mouth
Drug release	Pharmacopeia acceptability	Yes	Drug release affects drug efficiency and safety
Content Uniformity	Pharmacopeia acceptability	Yes	Variability in content uniformity will affect safety and efficiency. Content uniformity of ODTs is critical.

An overall risk assessment of the drug product formulation components was performed to determine which formulation components have a high risk of impacting the drug product CQAs. Table 3 shows us risk assessment results for formulation component effect on powder blend as a sample.

Table 3: Risk assessment of the formulation components.

Drug Product CQAs	Formulation Components
	Powder Blend
Size	No
Taste, odour	Low
Friability	High
Hardness	High
Disintegration time	Medium
Wetting time	Medium
Water absorption capacity	Medium
Drug release	High
Content uniformity	High

Since the detailed production processes were not set up during the risk assessment stage of the formulation development, risk analysis has been carried out on each and every formulation characteristic to achieve the optimized production process. Formulation variables of the powder mixture have been determined and effects of these variables on the critical quality parameters have been determined by the risk assessment (Table 4).

Table 4: Risk assessment of formulation variables.

Powder blend CQAs	Formulation Variables				
	Dispersant Type	Dispersant Amount	Lubricant Amount	Sweetening Agent	Particle size
Taste	Medium	Medium	Medium	High	Low
Bulk Density	Low	Low	Low	Low	High
Tapped Density	Low	Low	High	Low	High
Hausner ratio	High	High	Medium	Medium	High
Carr's Index	High	High	High	Medium	High

Applying QbD by Unit Operation

The purpose of each unit operation should guide evaluation of critical attributes (CQAs) with defining unit operations by specific output required and considering the order of unit operations. Figure 1 shows the unit operation steps, quality attributes of input and output materials and also all process parameters for all steps.

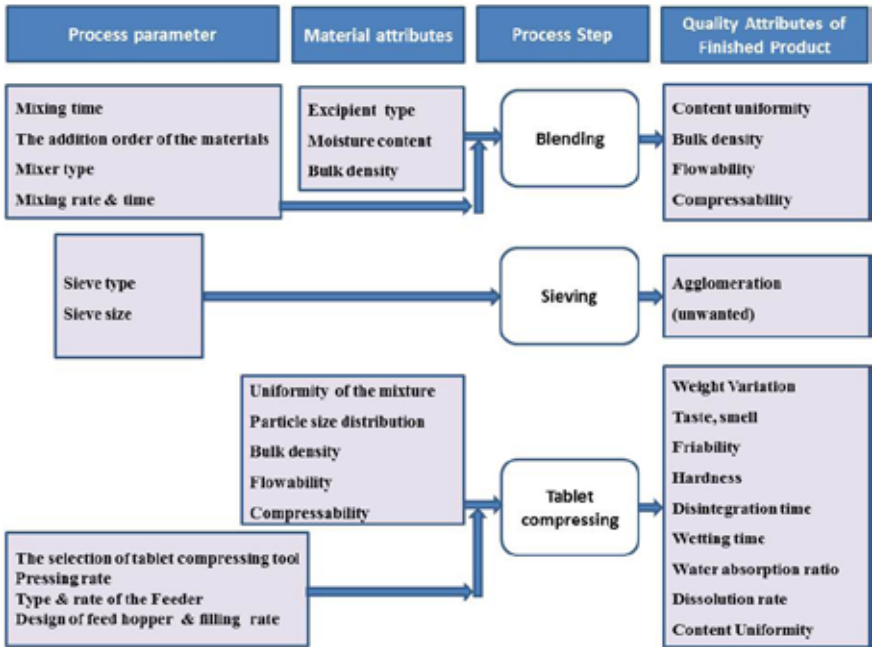

Figure 1: Unit operations map.

Risk Assessment

FMEA (failure modes and effects analysis) or use of a prioritization matrix (cause and effect matrix) is helpful in identifying the process inputs that impact on quality attributes. In some cases, a deeper dive into the driving forces at critical control points in the manufacturing process can yield a more fundamental understanding of sources of variation[18]. A risk assessment was performed and ten process parameters were evaluated using FMEA as a risk assessment tool to quantify the degree of risk associated with these materials and the design and process variables. As a part of the assessment, a system of ranking named risk qualification was established. The three rankings were severity (S), probability (P) and detectability (D) and are shown in Table 5. Severity (S) assesses the implications of a failure and how this failure may affect the quality of a product. The possibility of a failure is called the probability of occurrence, whereas detectability is the capability to detect failure modes. The S, P and D scores are multiplied to calculate a risk priority number (RPN) to list each risk according to its rank. Each score is given an

assessment point from one to five, and the multiplied RPN scores are classified as follows: Low (1-45), Moderate (46-90) and High (91-125). For a high RPN, the potential risks were deemed to have a critical adverse effect on the product quality[19].

Table 5: Ranking of severity (S), probability (P) and detectability (D).

SEVERITY		
Score	**Definition**	**Description**
1	Very low	Predicted to have no impact on product quality (quality within specifications).
2	Low	Predicted to have a minor impact on product quality (failure to meet specifications).
3	Moderate	Predicted to have a noticeable impact on product quality, but can be recovered.
4	High	Predicted to have a definite impact on product quality that may require rework.
5	Extreme	Predicted to have a severe impact on product quality and cause batch failure that is not recoverable.
OCCURRENCE		
Score	**Definition**	**Description**
1	Unlikely	Failure is unlikely to occur. Failure has never been seen but it is theoretically possible.
2	Rare	Failure is rare but has a remote probability. Failure has been seen once or twice.
3	Occasional	Failure infrequently occurs. Failure has been observed in several experiments.
4	Moderate / Probable	Failure potential is low. Failure has been observed in several experiments and may require in-process controls.
5	High/ Frequent	Failure is expected to occur regularly. Failure potential is high.
DETECTABILITY		
Score	**Definition**	**Description**
1	Always	Failure can be detected in all cases. Failure is clearly visible.
2	Regular	Failure can be detected almost every time.
3	Likely	Failure cannot be detected occasionally. Failure may be missed sometimes.
4	Low	Failure is probably not detected. Failure may be missed often.
5	Very low/ no detection	Failure cannot be detected/Failure cannot be detected with the available equipment or method.

Table 6 shows the risk score matrix, which is a part of FMEA.

Table 6: Risk assessment with FMEA.

Failure Mode	Failure Causes	Failure Effects	S*	P*	D*	RPN*	Precautions
Appearance	Die chose, colour chose,	Low patient compliance	3	6	5	**90**	Choosing appropriate parameters on effect the appearance on the tablets
Tablet size	Weighing wrong amount of powder mixture	Dissolution and disintegration rate are effected badly	8	3	9	**216**	Choosing appropriate tableting device and compression pressure.
Palatability	Using wrong amount of taste masking ingredient	Low patient compliance	10	3	9	**270**	Choosing appropriate taste masking excipient type and amount.
Friability	Compression pressure, lack of homogenous because of wide particle size distribution	Loss of API and other excipients Lack of content uniformity	10	4	10	**400**	Choosing appropriate compression pressure, excipient type and amount.
Hardness	Content of the formulation, compression pressure, compressing machine, binder amount, particle size	Delay in wetting and disintegration time, Effect the dissolution rate so bioavailability	8	3	10	**240**	Choosing appropriate compression pressure, excipient type and amount.
Disintegration time	High compression pressure, high tablet weight, disintegrant amount,	Effects the dissolution rate so bioavailability	10	3	10	**300**	Choosing appropriate compression pressure, excipient type and amount.
Wetting time	High tablet weight, compression pressure	Effects the disintegration and the dissolution rate so the bioavailability of the drug	8	3	10	**240**	Choosing appropriate compression pressure and tablet weight
Water Absorption Ratio	Content of the formulation, compression pressure, tablet weight	Effects the disintegration and the dissolution rate so the bioavailability of the drug	7	3	10	**210**	Choosing appropriate compression pressure and tablet weight
Dissolution rate	compression pressure, tablet weight, friability	Effects on the efficacy and safety of the drug	10	6	10	**600**	Choosing appropriate compression pressure and tablet weight
Content uniformity	Homogenous mixing, tableting rate, feeder rate	Effects on the efficacy and safety of the drug	10	6	10	**600**	Appropriate mixer, tableting and feeder rate

** S: Severity, P: Probability, D: Detectability and RPN: Risk priority number*

The potential CQAs of excipients required for development of ODTs were identified to have minimum disintegration time, water absorption capacity and wetting time with maximum hardness at friability not greater than 1%, also sweat taste for patient convenience.

In this study, CMAs (disintegrant type and amount) and CPPs (tablet compression pressure) were considered the inputs, and QTPP properties (hardness, friability, wetting time, water absorption ratio and disintegration time) were the outputs.

Evaluation of the Granulations and ODT Tablets

Alfuzosin hydrochloride granulations were prepared according to the following independent variables: disintegrant type (Ludiflash® or combination of mannitol and sodium starch glycolate (SSG)), disintegrant excipient % rate (30-80%) and tablets were pressed at pressures 500 or 1000 psi). The formulation variables and tablet compression pressures used are given in Table 7.

Table 7: Composition and compression pressure of ODT formulations.

Variable	Formulation					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Compressive strength	500	500	500	1000	1000	1000
Alfuzosin HCl (mg)	2,5	2,5	2,5	2,5	2,5	2,5
Ludiflash® (mg)	60	120	160	60	120	160
Avicel® (mg)	135,1	75,1	35,1	135,1	75,1	35,1
SSF* (mg)	2,4	2,4	2,4	2,4	2,4	2,4
Total weight (mg)	200	200	200	200	200	200
	F7	**F8**	**F9**	**F10**	**F11**	**F12**
Compressive strength	500	500	500	1000	1000	1000
Alfuzosin HCl (mg)	2,5	2,5	2,5	2,5	2,5	2,5
Mannitol+ SSG (mg)	60	120	160	60	120	160
Avicel® (mg)	135,11	75,1	35,1	135,1	75,1	35,1
SSF* (mg)	2,4	2,4	2,4	2,4	2,4	2,4
Total weight (mg)	200	200	200	200	200	200

**Sodium stearyl fumarate*

After blending all ingredients, bulk density, tapped density, Carr's index and Hausner ratio tests of prepared granulation formulations were carried out according to Eur. Pharm.[20-21] requirements.

The granules were compressed into tablets by using a single tablet punch press machine (Korsch, EK-0, Germany) using two different compression pressures (500 or 1000 psi). All the formulated ODTs were subjected to the following quality control tests.

Weight Variation

The weight variation test is carried out in order to ensure uniformity in the weight of tablets. The total weight of 20 tablets from each formulation is determined and the average is calculated. The individual weight of the each tablet is also determined to find out the weight variation.

Tablet Hardness

The hardness of tablet is an indication of its strength. Measuring the force required to break the tablet across tests it. Tablet hardness was measured, mean value and standard deviation was calculated (n=10).

Friability

Friability is the loss of weight of tablet in the container due to removal of fine particles from the surface. Tablets were weighed and placed in a standard Erweka® friabilator. The friabilator was operated at 25 rpm for 4 min, and the friability was then calculated as the percent loss in weight after the run[22].

Wetting Time and Water Absorption Ratio

For this purpose, a tablet was placed on a piece of tissue paper folded twice and kept in a small Petri dish (ID = 6.5 cm) containing 6 ml of water, and the time for complete wetting was measured. The wetted tablet was then reweighed to evaluate water absorption ratio. Water absorption ratio %, R was determined according to the literature[23-24].

In Vitro Disintegration Test

The *in vitro* disintegration test was performed according to the European Pharmacopoeia at 37±2°C in 900 mL of distilled water. One tablet was placed in each of the six tubes of the apparatus containing distilled water. A disk was added to each tube. The time required for the complete disintegration of the tablet until no mass remaining in the tube was measured. The disintegration time of three tablets in a single batch was determined, and the mean value and standard deviation was calculated[25].

Modelling and Optimisation

Design space can be described in terms of ranges of material attributes and process parameters, or through more complex mathematical relationships. It is generally determined through statistically designed experiment such as Design

of Experiment (DoE). This enables maximum information with minimum experimental trials. Design space is only for CPP or critical material attributes that has direct impact to product CQA. It can be established for each unit operation or spans a few unit operations or the entire process[26].

Experimental study data performed to create design space were evaluated with ANN and GEP modules in INForm program.

Software Tools

In this study, INForm V.5 ANN and INForm V.5 GEP (Intelligensys Ltd., UK) programs were used to develop predictive models and optimizes these models[27]. Whereas the task of establishing a central model is undertaken by the neural network element, genetic algorithms embedded in the software are used for optimization[28]. The Gene Expression Programming was used as an alternative to ANN for generating models to describe the linkages between the input and output parameters.

In our study, disintegrant type and amount and compression pressure were considered the inputs, and hardness, friability, wetting time, water absorption ratio and disintegration time were the outputs.

Training Software Tools Parameters

CPP and CMAs defined as variable factors and ANN and GEP modelling executed in INForm program by using the CQA results of the trials performed with these variable factors. Experimental data were analysed with the GEP and ANN to determine how to identify the optimum properties to achieve the optimum desired properties of the product formulation and/or treatment variables. In the programs, disintegrant type, disintegrant amount, Avicel® amount and compression pressure were considered the inputs; hardness, friability, wetting time, water absorption ratio and disintegration time were considered the outputs. Twelve formulations were used for model training. The test data selection was made using the "Smart Selection" method. The criterion for judging the models, fitness type was selected as Mean Square Error (MSE).

Because the training parameters influence the structure of the neural networks during the training process, the parameters in INForm V.5 was manipulated to optimize the predictability of the trained networks[10]. After trying various parameters, it was found that the parameters suggested in the ANN and GEP were suitable[29]. The ANN program study conditions are given in Table 8 and GEP program study conditions are given in Table 9. To validate the predictability of trained models, the nonlinear coefficient of determination was computed against the validation data set.

Table 8: INForm ANN study conditions for ODTs.

INForm ANN study conditions				
				INPUTS/ OUTPUTS
Model type: Neural Network		**Training parameters**		**Inputs**
Number of hidden layers (HL)	1	**Back-propagation parameters**		Dis. Exp. Type Dis. Exp. Rate Avicel Amount Comp. pressure
Current hidden layer (CHL)	1	Momentum	0.7	**Outputs**
Number of nodes (NN)	3	Learning rate	1000	Hardness Friability Disintegration time Wetting time Water abs. ratio
Transfer function	Asymmetric Sigmoid	**Targets**		
		Target epochs	0.0001	
		Target MS error	1000	
Output transfer function	Linear	Random seed		

Table 9: INForm GEP study conditions for ODTs.

INForm GEP study conditions				
Model type: Gene expression		**Fitness type: Mean Squared Error (MSE)**		**INPUTS/OUTPUTS**
				Inputs
				Dis. Exp. Type Dis. Exp. Rate Avicel Amount Compression pressure
No. of populations	10			**Outputs**
Population size	1000	Node weighting factor	0.5	Hardness Friability Disintegration time Wetting time Water absorption
No. of generations	200	Minimum operator nodes	3	
Headlength	7			
Number of genes	3			

Optimisation

After the training was completed, ANN and GEP recommended a set of conditions (formulation) at which the optimum levels for the quality attributes could be achieved.

Optimisation of ODTs was performed in this study using the INForm V.5 ANN and GEP. When the INForm ANN and GEP model were trained, the model was optimized with target values based on pharmacopeial and in-house specifications. Then, to find the formulation with the closest match to the optimised formulation, the best match feature of the program was used.

During optimization stage, each property weight value was specified as 10 to evaluate the importance of each critical parameter on a scale of 0 to 10, with 10 being the most important.

Optimisation parameters are:

Number of Populations: 1

Population Size: 100

Number of Iterations: 100

Mutation SD: 0.1

Random Seed: 1

RESULTS AND DISCUSSION

Evaluation of Alfuzosin Hydrochloride Orally Dispersible Tablets

-Evaluation of blends before compression

Results that belonged to the pre-compressing tests required for determination of flow properties such as bulk density, tapped density, Carr's (Compressibility) index and Hausner ratio of powder blends of ODT formulations are given in Table 10.

Table 10: Flow characteristics of powder blends of ODT formulations.

Parameter	Powder Blend								
	F1/F10	F2/F11	F3/F12	F4/F13	F5/F14	F6/F15	F7/F16	F8/F17	F9/F18
Bulk density (g/mL)	0.42	0.43	0.52	0.53	0.51	0.50	0.51	0.50	0.55
Tapped density (g/mL)	0.57	0.62	0.7	0.73	0.7	0.65	0.72	0.67	0.75
Hausner ratio	1.36	1.44	1.35	1.38	1.37	1.30	1.41	1.34	1.36
Carr's index	26	31	26	27	27	23	29	25	27

-Post compression evaluations

Results of the test applied to the tablets prepared are given in the Table 11.

Table 11: The characteristics of ODT tablets prepared.

Code	Hardness			Friability (%)	Weight variation (g)	Disintegration time (min)		Wetting time (s)	Water absorption ratio (%)
	Mean Value (N)	SD (±)	RSD %			Mean Value (min.)	SD (±)		
F1	10.8	0.46	4.29	0.15	0.2001±0.001	1.02	0.08	0.32	55.5
F2	11.7	0.51	4.32	0.19	0.1993±0.0016	1.20	0.03	0.27	54.6
F3	8.02	1.01	12.53	0.55	0.1991±0.0009	0.43	0.03	0.17	47.7
F4	9.82	0.82	8.37	0.41	0.2005±0.0011	0.30	0.02	0.35	59.0
F5	8.7	0.25	2.87	0.37	0.2017±0.0011	0.22	0.03	0.18	56.0
F6	8.9	0.1	1.08	0.55	0.2016±0.0012	0.34	0.04	0.28	57.8
F7	16.5	1.36	8.24	0.12	0.1995±0.0011	4.02	0.12	0.58	24.7
F8	15.5	0.45	2.91	0.16	0.2016±0.0016	1.52	0.05	0.41	45.0
F9	14.58	0.22	1.49	0.21	0.2019±0.0009	1.25	0.10	0.44	40.4
F10	17.95	0.93	5.18	0.34	0.2009±0.0012	3.05	0.20	1.10	41.6
F11	16.42	0.81	4.92	0.31	0.2013±0.0015	1.50	0.09	0.49	38.1
F12	16.9	1.03	6.09	0.40	0.2012±0.0014	3.07	0.08	1.10	40.0

Optimisation with GEP and ANN Programs

Models having been derived based on the ANOVA test results of the program were tested (See Table 12 and 13).

Table 12: The R^2 values calculated between ODTs included alfuzosin hydrochloride and anticipated values.

INForm ANN - ODT Attributes / R^2 values	
Outputs	**R^2**
Hardness (N)	96.32
Friability (%)	97.51
Wetting time (s)	91.65
Water absorption ratio (%)	97.38
Disintegration (s)	96.92
Dissolution (%)	98.19

Table 13: The R^2 values calculated between ODTs included Alfuzosin hydrochloride and anticipated values.

INForm GEP - ODT Attributes / R^2 values	
Outputs	**R^2**
Hardness (N)	0,97
Friability (%)	0,98
Wetting time (s)	0,99
Water absorption capacity (%)	0,92
Disintegration (s)	0,97
Dissolution (%)	0,98

ANN model gave information about which formulation is similar to the optimised formulation. Formulation 2 has the highest similarity to the optimised formulation at the rate of 60.01% (Table 14).

Table 14: The comparison of optimised Formulation and Formulation 2, the best match formulations according to the ANN model.

	Optimised Formulation	**Formulation 2**
Superdisintegrant type	Ludiflash®	Ludiflash®
Superdisintegrant (%)	30.00	60.00
Avicel® amount (mg)	35.00	75.00
Compression pressure (psi)	604.67	500.00
Hardness (N)	11.13	11.70
Friability (%)	0.16	0.19
Wetting time (s)	27.19	27.00
Water absorption ratio (%)	44.40	54.60
Disintegration (s)	48.78	80.00
Dissolution (%)	74.77	49.57

GEP model gave information about which formulation is similar to the optimised formulation. Formulation 3 has the highest similarity to the optimised formulation at the rate 89.64% (Table 15).

Table 15: The comparison of optimised formula and Formulation 3, the best match formulations according to the GEP model.

	Optimised Formulation	Formulation 3
Disintegrant type	Ludiflash®	Ludiflash®
Disintegrant amount (%)	69.36	80.00
Avicel® amount (mg)	35.00	35.00
Compression pressure (psi)	547.00	500.00
Hardness (N)	9.45	8.20
Friability (%)	0.34	0.55
Wetting time (s)	21.91	17.00
Water absorption ratio (%)	48.62	47.70
Disintegration (s)	42.46	43.00
Dissolution (%)	84.87	93.97

With respect to the FMEA result and prior knowledge and experiences, taste, friability, hardness, disintegration time, wetting time and water absorption ratio were classified as CQAs, blending time and rate, sieve size and compression pressure were classified as CPPs. Disintegrant type (Ludiflash® or combination of mannitol and sodium starch glycolate), disintegrant % rate (30% - 80%) determined as CMAs. Whereas certain risk scores state that the appearance were not considered CQAs.

A total number of nine formulations were prepared and their powder blend characteristics were evaluated. For the flow characteristic of a powder mixture to be considered good, the Hausner ratio should be less than 1.25[20]. As shown in Table 10, the Hausner ratio for all the formulations was greater than 1.25, that's why the flow properties were considered as not so good. According to results formulations that were prepared had bed compressibility. In order to decide which formulations could be eliminated, formulations (Table 7) were compressed at 500 or 1000 psi and disintegration time was evaluated.

The disintegration time varied depending on the formulation components and the compression force, although all the formulations except F7, F10 and F12 complied with the European Pharmacopoeia limits[25]. The hardness values and compression forces of tablet formulation were directly proportional, as expected and F7, F10 and F12 formulations having the highest hardness value and the longest disintegration time. The friability values of tablet formulations were below 1%.

All the tablets from each formulation passed weight variation test, as the % weight variation was within the pharmacopoeia limits of ±7.5% of the weight. The weight variation in all the prepared formulations was found to be 201±0.9

and 199±0.9 mg, which was in pharmacopoeia limits[31].

According to analyses conducted on tablets, experimental data was formed then evaluated *via* software uses artificial intelligence to perform mathematical modelling and optimization studies.

Based on the evaluation of the ANN data, an ODT formulation was recommended. The suggested "optimised formulation" contained 2.5 mg alfuzosin hydrochloride, 30% Ludiflash®, and 35 mg Avicel® and with a compression force of 605 psi. The program also provided "outputs" for the formula that it suggested. Accordingly, the predicted formulation properties of the optimised formula were 48.7 s for disintegration time, 11.1 N for hardness, 27 s for wetting time, 44% for water absorption ratio and 0.16% for friability.

The ANN model also provides us information about which formulation best fit the optimized formula. F2 was found to be the most similar to the optimized formula with a 60 % similarity by the ANN model.

Based on the evaluation of the GEP data, an ODT formulation was recommended. The suggested "optimised formulation" contained 2.5 mg alfuzosin hydrochloride, 69% Ludiflash®, and 35 mg Avicel® with a compression force of 547 psi. The program also provided "outputs" for the formula that it suggested. Accordingly, the predicted formulation properties of the optimised formula were 42.4 s for disintegration time, 9.45 N for hardness, 21.9 s for wetting time, 48.6% water absorption ratio and 0.33% for friability.

The GEP model also provides us information about which formulation best fit the optimized formula. F3 was found to be the most similar to the optimized formula with an 89.6% similarity by the GEP model.

It has been shown that both models proffer using Ludiflash®, which contains Crospovidon, a polymer used in other studies [32, 33] as disintegrant within the ODT formulations and found suitable, in the tablet formulation to better quality properties and using it even in low amounts like 30 % of total tablet weight will provide sufficient effect to maintain convenient disintegrating time and other quality attributes.

Even though there are several studies[33, 34] conducted to show the convenient of using co-processed disintegrant or different disintegrant polymer forms, in this study using Artificial Intelligence Modelling methods aided to develop formulation with a novel approach.

CONCLUSION

Through our study, multiple experiments with direct compressed alfuzosin hydrochloride ODT were evaluated in order to understand relationships between input attributes and outputs were obtained.

In these experiments, data was acquired on how the formulas and processes inputs affect output variables. Evaluating experimental results via GEP and/or ANN modelling software show the multivariate and complex relations among all the variables that determined as critical before, at the same time. Formulation and process knowledge on how the critical parameters affect the quality attributes were increased. Also, these modelling studies helped to develop models based on the known data results to estimate the unknown results for the data sets by optimization ability.

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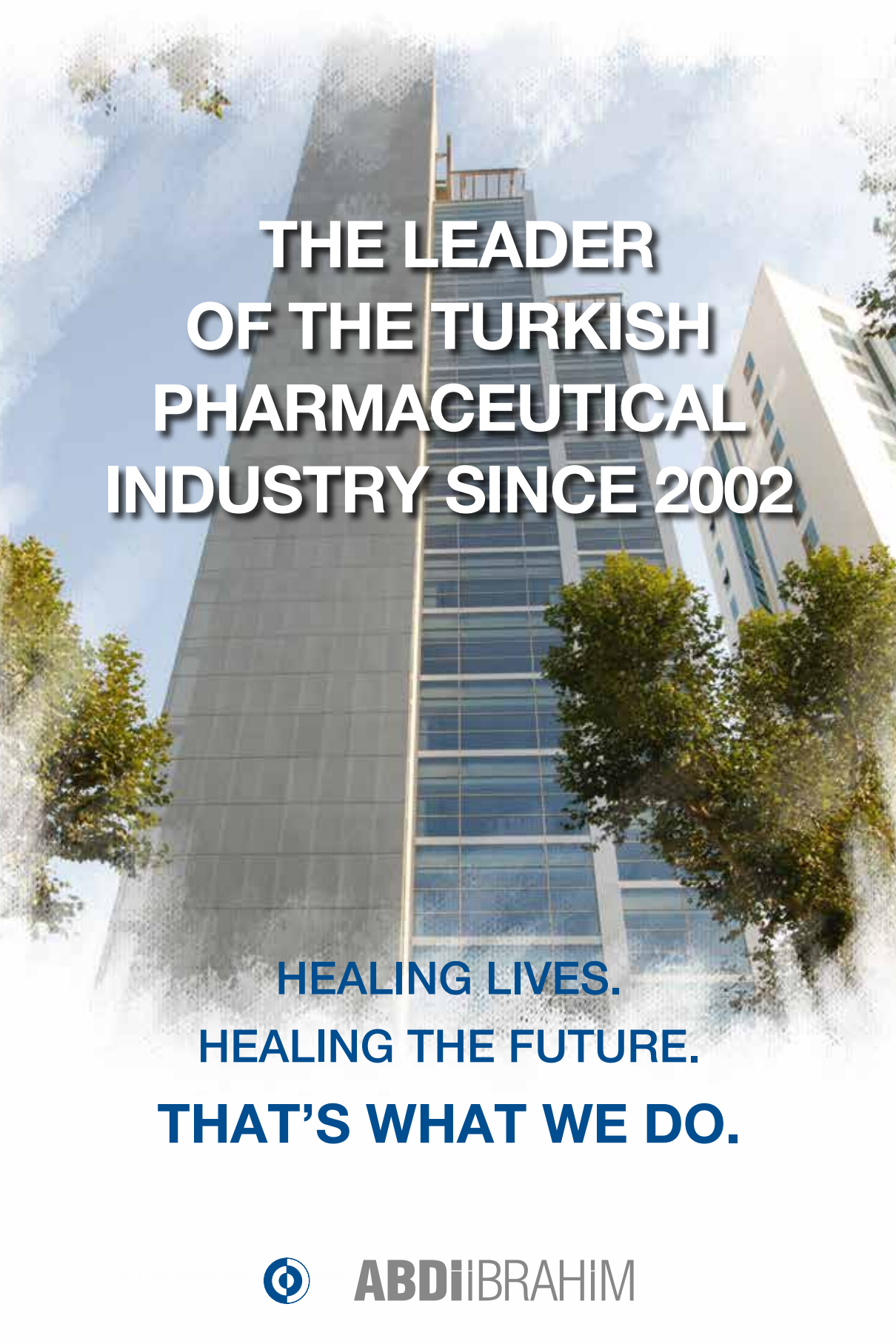
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Formulation Development and Characterization of Oxcarbazepine Microemulsion for Intranasal Delivery

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ABSTRACT

The objective of this study was to develop novel intranasal microemulsion containing oxcarbazepine (OXC) for treatment of epilepsy. Optimized ratio of Tween 80: Polyethylene glycol and isopropyl myristate was selected after developing pseudoternary phase diagrams and microemulsions were prepared. The prepared microemulsions were characterized for drug content, pH, particle size, polydispersity index, zeta potential, conductivity, viscosity and *in vitro* release. *Ex vivo* permeation study for selected microemulsion was performed through sheep nasal mucosa. Further pharmacodynamic performance was evaluated in mice by electrically induced seizures. It was found that selected microemulsion was transparent with average globule size of 20.5 nm and cumulative percentage drug permeated was 95.60 %. Pharmacodynamic evaluation of selected formulation also indicated lesser intensity of seizures with low dose in mice in comparison to oral suspension of OXC. OXC intranasal delivery system is an effective alternate therapy for treatment of epilepsy.

Keywords: Oxcarbazepine, intranasal, microemulsion

INTRODUCTION

Several methods have been reported in the literature to enhance the drug penetration across biological membranes[1]. Nasal drug delivery is an alternate route to oral and parenteral route for the drug to reach systemic circulation. As nasal drug delivery shows various benefits in comparison to other forms of drug deliveries.

Nasal cavity is lined by vascularized epithelium which provides larger surface area useful for drug absorption. It has low enzymatic activity in contrast to the di-

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gestive system. It bypasses hepatic first pass metabolism. So there is negligible irritation of gastrointestinal membrane[2-3]. Nasal drug delivery can be preferred over other routes of drug delivery as it is non-invasive, convenient method with better patient compliance, easy and cost effective[4-5]. Nasal drug delivery also offers advantage of transporting the drugs to brain by detouring the blood brain barriers[6].

Microemulsions (ME) are clear, thermodynamically stable and isotropic mixtures[7] (Oil, water and surfactant, mostly along with cosurfactants). ME should be kept under proper storage conditions[8]. There are three types of ME microstructures. They are: Micellar (oil in water), inverted micellar (water in oil) and bicontinuous structure. ME is a new approach to sparingly water soluble drugs, in order to enhance their dissolution and improve bioavailability.

Epilepsy is one of the most common and devastating neurological disorders which is estimated to have a worldwide prevalence of about 0.5–1% [9]. There are several antiepileptic drugs currently available to control and suppress seizures. However, despite the ongoing development of new pharmacological therapies, more than 30% of the patients do not become seizure free mainly due to the pharmacoresistance phenomena[10].

In order to ameliorate the antiepileptic drug regimen, various strategies of administration have been explored. An intranasal microemulsion is one of the advanced drug delivery option, which can be given orally, topically and through nasal cavity as aerosol[11].

Intranasal ME will transport the drug from nose to brain at a very faster rate. By improved distribution and dissolution of drug within the brain, one can assume reaching higher levels of therapeutic index along with the benefit of reduced side effects, low dosages and also reduction in the cost of therapy[12].

Intranasal microemulsion formulations have been developed for a number of drugs such as Quetiapine fumarate[13], Fexofenadine[14], Diazepam, Lorazepam, Alprazolam[15], Eucalyptus oil[16], Ibuprofen[17] and Zolmitriptan[18].

Oxcarbazepine (OXC) is an anticonvulsant and mood stabilizing drug, used primarily in the treatment of epilepsy and is also used to treat anxiety / mood disorders. It is a derivative of carbamazepine. Chemically it is 10, 11-dihydro-10-oxo- 5H-dibenz (b,f)azepine-5-carboxamide (As shown in Figure 1). It is poorly soluble in water (308 mg/L) and has a partition coefficient of 1.31. It belongs to iminostilbene category of antiepileptic's and act on convulsions by post tetanic potentiation of synaptic transmission, also act on neuropathy by sodium channel blockade and calcium channel blockade mechanism and act on bipolar disorder by decreasing abnormal electrical activity in brain[19].

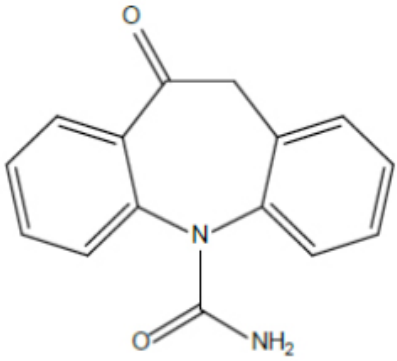

Figure 1: Structure of oxcarbazepine.

The objective of this investigation is to develop intranasal OXC and to compare with oral formulation available in market.

METHODOLOGY

Materials

OXC was obtained from Aurobindo Pharma Ltd., Hyderabad, India. Sunflower oil from local market, Isopropyl myristate, Tween 20, Tween 80, PEG 400, PEG 600, glycerol, propylene glycol, oleic acid, methanol, sodium hydroxide & potassium dihydrogen phosphate were obtained from S.D. Fine-Chem Ltd., Mumbai. Dialysis membrane was procured from Himedia, Mumbai. All the chemicals were of analytical grade and purchased commercially. Double distilled water was used throughout the study.

Software used: Ternary phase diagram (CHEMIX School 3_60).

Methods

Solubility Studies

The solubility of OXC in various components (oils, surfactants and cosurfactants) was determined by adding an excess of drug to 25 ml conical flask, containing 10 ml of selected vehicle and vortexed for half an hour and placed on a rotary shaker for 48 hours at room temperature. Then contents were centrifuged (REMI R-8C) at 3000 rpm for 5 minutes. The supernatant was filtered through a membrane filter (0.45µm) and the drug concentration in filtrate was determined by UV-Visible (UltraViolet) spectroscopy (Thermo Fisher Scientific Model Evolution 201 series). The oil, surfactant and cosurfactant that showed high soloubility of OXC was used in preparation of microemulsions.

Preparation of Pseudoternary Phase Diagram[20]

Pseudoternary phase diagrams were constructed to obtain the appropriate ratio of surfactant: cosurfactant which can result in to large existence of microemulsion area. They were constructed using water titration method. Surfactant (Tween 80) and cosurfactant (PEG 600) were mixed (Smix) in different weight ratios (1:1, 2:1, 4:1) and represented as A-series, B-series and C-series respectively. Oil (isopropyl myristate) and Smix (Tween 80 and PEG 600) were mixed thoroughly in different weight ratios from 1:8 to 8:1 in different test tubes and diluted with distilled water in a drop wise manner till it changed from opaque to transparent (composition shown in Table 1) . The concentrations of components were recorded in order to complete pseudoternary phase diagrams, and then the contents of oil, surfactant, cosurfactant and water at appropriate weight ratios were selected based on these results.

Table 1: Formulation composition of microemulsions

Formulation code	Smix (Tween 80 and PEG 600)	Oil and Smix
A-Series, A1- A8	1:1	1:8 to 8:1
B-Series, B1 - B8	2:1	1:8 to 8:1
C-Series, C1 - C8	4:1	1:8 to 8:1

By joining the change points the boundaries of phases formed were obtained in the phase diagrams. All samples exhibiting a transparent and homogenous state were assigned to a microemulsion area, a monophasic area, in the phase diagram. The pseudoternary phase diagrams were constructed by using CHEMIX School 3_60 software (As shown in Figure 2).

Preparation of Microemulsion

The OXC microemulsion was prepared by phase titration method employing Isopropyl myristate (IPM) as oil, Tween 80 as surfactant and PEG 600 as cosurfactant.

Preparation of Microemulsion with 1:1, 2:1 And 4:1 Ratio of Smix

Accurately weighed 50 mg of the drug was added to test tube containing Smix (Tween 80 and PEG 600) in 1:1 ratio. The mixture was shaken on a cyclo mixer until the drug gets properly mixed. Oil (Isopropyl myristate) was then added to the Smix and again shaken for about 10 min. The mixture was diluted with distilled water in a drop wise manner under constant stirring till a transparent microemulsion was achieved.

IPM and Smix were mixed thoroughly in different weight ratios from 1:8 to 8:1

(A1 to A8). The pseudoternary phase diagrams were constructed to know the area of the microemulsion formed. Similarly microemulsions with 2:1 and 4:1 ratios of Smix and varying ratios of Oil: Smix (B1 to B8 and C1 to C8 respectively) were prepared and pseudoternary phase diagrams were constructed.

Characterization of Microemulsion

For the selected three formulations viz., A2, A5 and B3 different characterization tests were done. These three formulations were selected based on transparency, viscosity and amount of water that can be incorporated. C-series of formulations resulted in microemulsions having high viscosity, turbidity and few resulted in gel like consistency after addition of water. These are the reasons for not selecting C-series (Smix 4:1) for further studies.

Drug Content

The drug content of microemulsion formulation was determined by dissolving 1 ml of the formulation in 10 ml of methanol. After suitable dilutions with methanol, absorbance was determined using the UV-Visible spectrophotometer keeping blank microemulsion as control at wavelength 256 nm.

pH Determination

The pH values of the microemulsions were measured by a pH meter (Digisun Electronics, India) at ambient temperature with glass electrode.

Particle Size Distribution, Polydispersity Index (PDI), Zeta Potential And Conductivity

Physical characteristics of microemulsion (particle size distribution, polydispersity index, zeta potential and conductivity) were determined by using Dynamic light scattering (DLS) method using a zetasizer (Horiba SZ-100Z, Japan).

Viscosity Measurement

Microemulsions are generally low viscosity systems. The viscosity of prepared microemulsion was measured using Brookfield viscometer (Brookefield viscometer LVDV-E, US).

In vitro Release Studies

Preparation of Calibration curve of OXC in 40% v/v PEG 400 plus phosphate buffer pH 6.4

Accurately weighed 10 mg of drug was dissolved in 100 ml of 40% v/v PEG 400 + phosphate buffer pH 6.4 which gives stock solution of 100 µg/ml.

From this stock solution aliquots of 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, 3.5 ml , 4 ml, 4.5 ml were pipetted out into a series of 10 ml volumetric flasks and

make up to mark with phosphate buffer which gives 5, 10, 15, 20, 25, 30, 35, 40, 45 µg/ml respectively.

The absorbance of the resulting solution was then measured at 256 nm using UV spectrophotometer. The calibration curve was obtained by plotting Absorbance vs. Concentration in µg/ml (Figure 3).

In vitro Release Studies

Based on characterization results three formulations were selected i.e., A2, A5 and B3 for *in vitro* release studies. The composition of selected formulations is shown in Table 2. The *in vitro* release study was carried out using Franz diffusion cell (Fabricated locally). The donor compartment was open at the top and was exposed to atmosphere. The dialysis membrane with molecular weight in the range 12,000 to 14,000 (Himedia, Mumbai) was previously soaked for 24 h in phosphate buffer pH 6.4. The donor and receptor compartments were held together using a clamp. The receptor compartment contained 13 ml of 40% v/v PEG 400+ phosphate buffer pH 6.4 and stirred with a magnetic capsule operated by a magnetic stirrer (REMI 2MLH, India). The temperature was maintained at 37 ± 0.5 °C and the receptor compartment was provided with a sampling port. Samples were collected at preset time points. At each sampling time, 3 ml of sample was removed using a syringe with syringe filter and replaced with fresh 40% v/v PEG 400 in phosphate buffer pH 6.4[21].

The concentration of drug was determined using a UV-Visible spectrophotometer at a wavelength of 256 nm. The percentage drug released was calculated and plotted against time (Figure 4).

Ex vivo Permeation Studies

Two formulations were selected for permeation study based on *in vitro* release studies. The freshly excised sheep nasal mucosa, except the septum part, was collected from the slaughter house. The membrane was kept in 40% v/v PEG 400 + phosphate buffer pH 6.4 for 15 min to equilibrate. The superior nasal concha was identified and separated from the nasal membrane[22]. The excised superior nasal membrane was mounted on a Franz diffusion cell[23]. Franz diffusion cell used for *ex vivo* permeation studies had a diameter of 2 cm and mucosa of thickness 0.2 ± 0.01 mm. The receptor compartment was filled with 14 ml of diffusion media. Diffusion media was continuously stirred with a Teflon-coated magnetic bar at a constant rate, in a way that the nasal membrane surface just flushes the diffusion fluid[24].

Two ml of OXC microemulsion was placed in the donor compartment of Franz diffusion cell. Samples were collected at preset time points and analyzed using

U.V spectrophotometer. Each sample removed was replaced by an equal volume of diffusion media (3 ml).

Each study was carried out for a period of 3 h, during which the drug in the receptor compartment (μg/ml) across the sheep nasal membrane was calculated at each sampling point.

The cumulative amount of OXC permeated through mucosa was determined by the following equation:

$$Q_n = \frac{C_n \times V_\circ + \sum_{i=1}^{n-i} C_i \times V_i}{S}$$

Where C_n is OXC concentration of receptor medium after each sampling time, C_i is oxcarbazepine concentration for i sample, $V_\circ$ and V are the volumes of receiver solution and sample respectively, and S is the effective diffusion area[25].

Pharmacodynamic Studies

Maximal Electroshock: Mice weighing about 25 gm and exhibiting clear hind limb extension phase during electrically induced convulsions were included in the present study. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC/SUCP/03/2014). Mice were divided into 3 groups (n=6). The first and second groups were treated orally with OXC suspension (0.5% Na CMC) and intranasally [23] with OXC microemulsion respectively containing OXC equivalent to 13.5 mg/kg body weight (using a micropipette attached with low density polyethylene (LDPE) tubing, having 0.1 mm internal diameter at the delivery site). For intranasal administration, 60 μl (0.2542 mg drug) of microemulsion was instilled equally divided in both the nares of mice. The third group was not subjected to any treatment, served as control. Electroconvulsions were produced by applying current (50 mA, 0.2 s) through ear clip electrodes using electroconvulsiometer after 30 min of administration of formulations and different phases of seizures were measured.

Briefly after application of current an immediate severe tonic phase (E phase) was observed which was characterized by maximal extension of the anterior and posterior legs. At the end of tonic phase, clonic phase starts which was characterized by paddling movement of the hind limb and shaking of body. During stupor phase which was observed after tonic and clonic phase mice remained silent without any movement. Recovery time was recorded as total time from starting of tonic phase till animal regains its normal movement (Figure 8).

RESULTS AND DISCUSSION

It is estimated that more than 98% of all small molecules and nearly 100% of large molecular weight drugs systemically delivered to the central nervous system (CNS), either by oral or intravenous routes, do not readily cross the blood brain barrier and reach the brain parenchyma at pharmacologically active concentrations[26].

In the light of the current knowledge, drug transport across the nasal mucosa into the CNS depends on a variety of factors that can range from the physicochemical properties of the drug to the formulation design and physiological conditions at the absorption site[27-28].

Solubility Studies

The solubility of the drug was determined in each component of microemulsion (oils, surfactants and cosurfactants) and was reported. Based on absorbance values (qualitative) oil, surfactant and co surfactant was selected.

Oils - IPM > Oleic acid > Sunflower oil

Surfactants – Tween 80 > Tween 20 > Cremophor EL

Co surfactant – PEG 600 > PEG 400 > Isopropyl alcohol > Glycerol > Propylene glycol

Depending on solubility results isopropyl myristate was selected as oil for preparation of microemulsion, tween 80 showed good solubility for OXC and previous studies have reported improved nasal absorption[29]. Thus tween 80 was selected as surfactant and based on solubility results PEG 600 was selected as cosurfactant. The water solubility of the drug was found to be 308 mg/ L.

Microemulsions were prepared by the spontaneous emulsification method (phase titration method) and can be depicted with the help of phase diagrams.

Preparation of Pseudoternary Phase Diagram

The components that showed maximum solubility were further optimized using pseudoternary phase diagrams as shown in Figure 2. The zone of microemulsion was obtained.

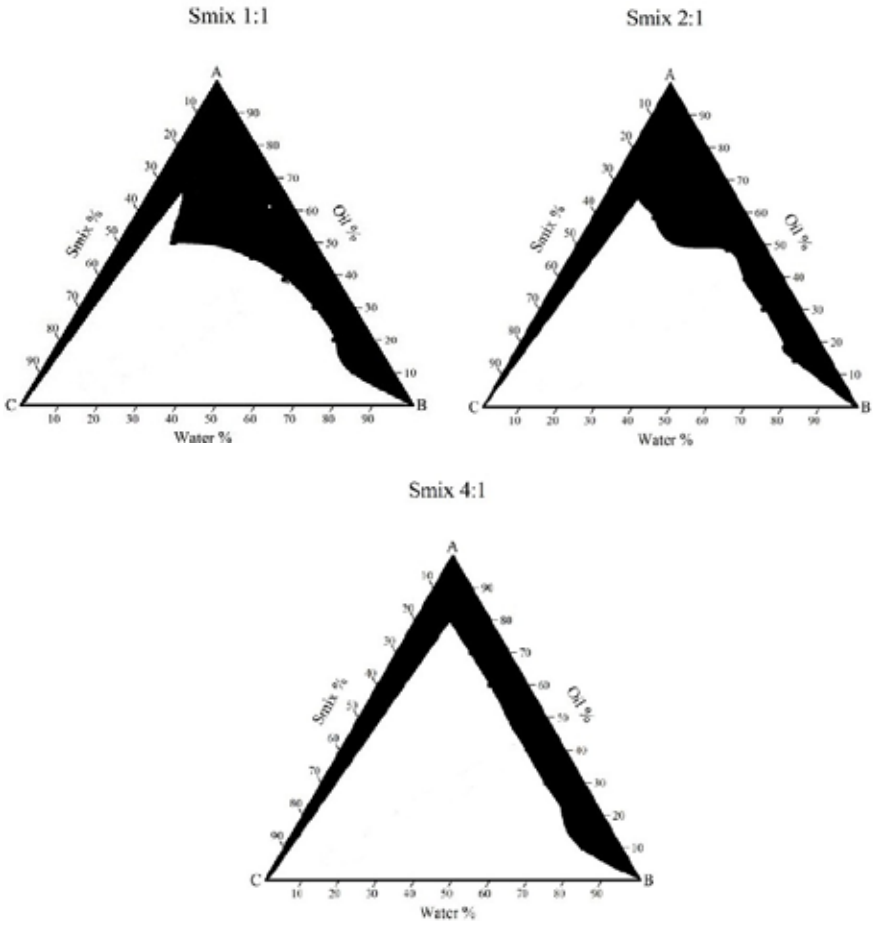

Figure 2: Pseudoternary phase diagram using isopropyl myristate as oil, tween 80 as surfactant, polyethylene glycol 600 as cosurfactant and water (Tween 80: PEG 600 = 1:1, 2:1, 4:1).

Preparation of Microemulsion

Eight formulations (1:8 to 8:1 ratio) from each ratio of Smix (1:1, 2:1 and 4:1) were prepared and the selected formulations were characterized thoroughly. Highest microemulsion area was obtained with ratio of 1:1, 2:1 and thus selected for further studies.

The OXC loaded microemulsion formulations were prepared as per the compositions shown in Table 2. Microemulsion systems were obtained by mixing oil, surfactant and cosurfactant together and adding appropriate quantity of OXC and adding precisely distilled water drop by drop to these oily phases with continuous stirring at ambient temperature. The final concentration of OXC in microemulsion systems was 5 mg/ml. Microemulsions have several specific physic-

ochemical properties such as transparency, optical isotropy, low viscosity[30]. The formulations having these specific physicochemical properties were selected for characterization, *in vitro* release and *ex vivo* permeation studies.

Table 2: Formulation composition of selected microemulsions.

Formulation code	OXC (mg)	Smix (%)	IPM (%)	Water (%)
A2	50	50	14.28	35.72
A5	50	38.46	48.07	13.47
B3	50	50.84	25.42	23.74

Characterization of Microemulsion

Drug content percentage of all the three selected formulations was found to be 99.25 ± 1.30. The pH was found to be 5.47 ± 0.42. Viscosity of all formulation was found to be 60.53 ± 5.93 cps. Physical characteristics of microemulsion (particle size distribution, polydispersity index, zeta potential and conductivity) were shown in Figures 3 & 4.

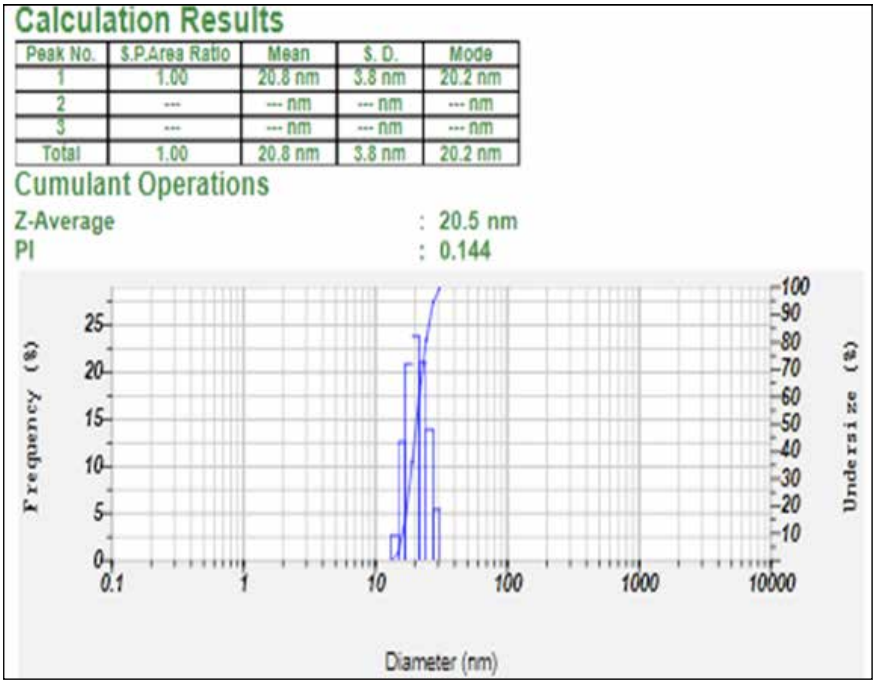

Figure 3: Particle size measurement of Formulation A2.

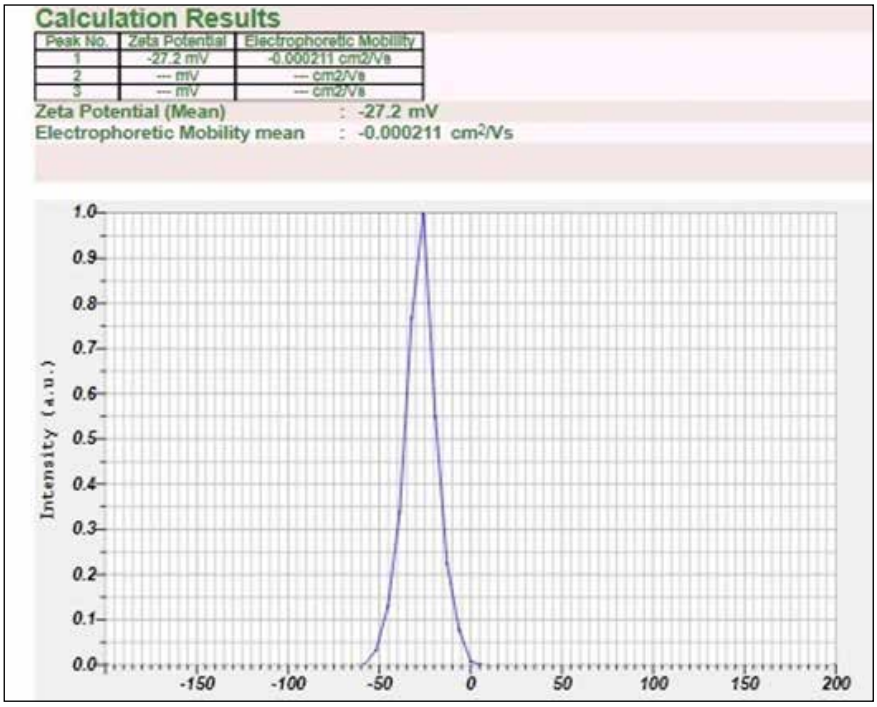

Figure 4: Zeta potential measurement of Formulation A2.

Result of globule size indicated that smallest globule size was obtained with formulation A2 with poly dispersity index 0.144 which is close to zero, indicating that the prepared microemulsion had uniform globule size and thus it was selected for further studies as faster permeation is expected when the globule size is small. Zeta potential was negative which indicated the stability of formulations as there were less chances of globules aggregation. The conductivity of the results confirmed the formation of solution type of microemulsion with water in continuous phase.

Calibration curve of Oxcarbazepine in 40% v/v PEG 400 + phosphate buffer pH 6.4:

Calibration curve of OXC in 40% v/v PEG 400 + phosphate buffer pH 6.4 was shown in Figure 5 and it was found to be linear.

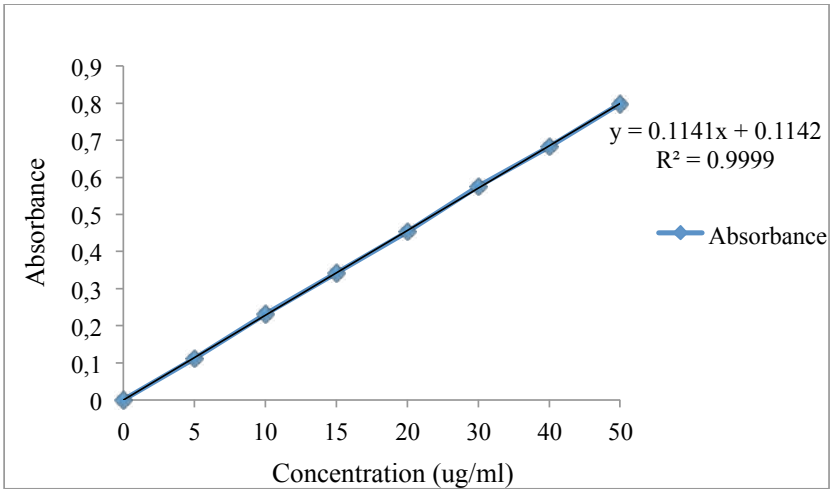

Figure 5: Calibration curve of OXC in 40% v/v PEG 400 + phosphate buffer pH 6.4

***In vitro* release studies:** The cumulative percentage of drug release after 210 min was found to be maximum with formulation A2 (98.65 %). *In vitro* release profile of OXC was shown in Figure 6. The formulation with more amount of water has shown maximum percentage drug release compared to other formulations.

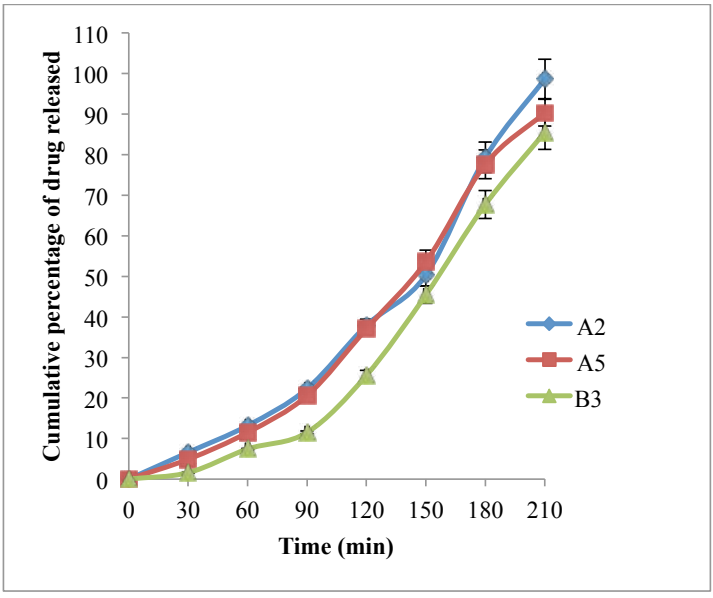

Figure 6: *In vitro* release profile of OXC.

***Ex vivo* permeation studies:** Although human nasal mucosa would be the ideal substrate for nasal permeation studies, its limited availability has made to use suitable alternative. It was reported[31] that the sinus anatomy (including the placement of nasal cavity, turbinates, frontal and maxillary sinuses) in sheep is comparable to humans. Histology of the sheep's nasal mucosa is also identical to that of humans[32]. Hence e*x vivo* permeation study was performed by using sheep nasal mucosa for optimized formulations A2 and A5. The cumulative percentage of drug permeated after 210 min was found to be maximum with formulation A2 (95.60 %) and formulation A5 shown 81.25 %. *Ex vivo* permeation profile of OXC from sheep nasal mucosa was shown in figure 7.

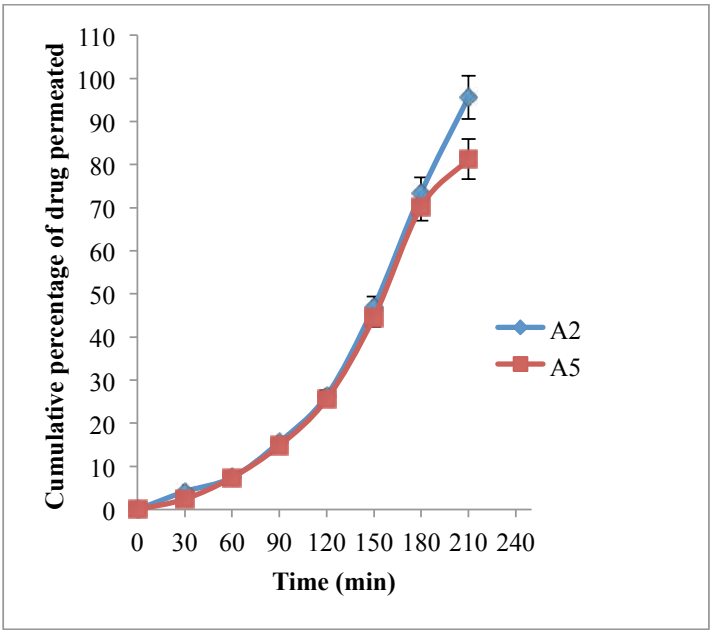

Figure 7: *Ex vivo* permeation profile of OXC through sheep nasal mucosa.

Pharmacodynamic studies

The antiepileptic activity was assessed by observing the extent of different phases of seizures including duration of seizures, extension phase, clonus phase and stupor phase and results were represented in Figure 8 and different phases in Figure 9. The results clearly indicated lesser intensity of seizures and rapid recovery from seizures in mice treated with intranasal OXC microemulsion.

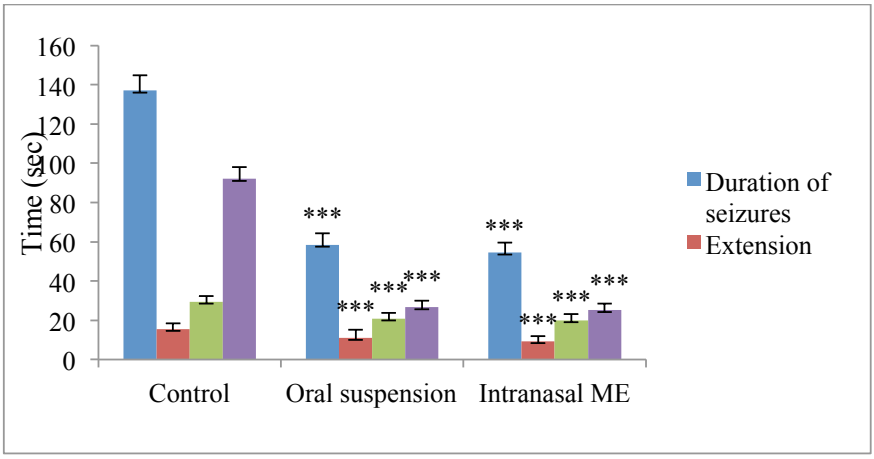

Figure 8: Duration of seizures, extension, clonus and stupor phase for two treatments of oxcarbazepine - OXC oral suspension and OXC microemulsion (IN) where, *** indicates significant difference in comparison to control ($p < 0.05$).

Tonic phase

Clonic phase

Stupor phase

Figure 9: Different phases of seizures in mice.

The intranasal OXC microemulsion demonstrated lesser intensity of seizures which may be due to larger extent of selective nose to brain delivery of drug in comparison to oral suspension of OXC. This may help in decreasing the dose and frequency of administration of drug and may possibly maximize therapeutic benefits and may also reduce the cost of therapy.

CONCLUSION

In comparison to oral formulation, intranasal microemulsion of OXC was shown significant difference in antiepileptic activity. However detailed animal study followed by thorough clinical trials is required to establish clinical safety and efficacy of this formulation.

CONFLICT OF INTEREST

There is no conflict of interest between the authors of the article.

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The Role of Pharmacists in Kırklareli (Turkey) as Primary Health Care Providers

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ABSTRACT

This study was carried out in Kırklareli in 2005. The aim was to investigate the role of pharmacists as primary health care providers. In 63.5% of the total number of 55 pharmacies taking part in the research, there was no health care service within a 150 meter range. The remaining pharmacies, constituting 36.4%, did have health care services within a 150 meter range. These were in the proximity of either village clinics (55%), state hospitals (35%), or private hospitals (10%). 51.9% of the pharmacists are female and 48.1% male, and usually, they employ 1 or 2 assistants; only 1.9% do not employ an assistant. The assistants are between the ages of 20-29, and most of them are high school graduates. 27.4% of them work 10 hours a day, and 43.5% of them work 11 hours a day. 23.3% of the medicine sold in pharmacies in the most recent week of the study was unprescribed. 84% of the pharmacists told the patient to inform them of any adverse reactions and/or side effects. In the case of adverse reactions and/or side effects, 57.7% of patients informed the pharmacist before consulting a doctor. 100% of the pharmacists guided the patient to TÜFAM (The National Pharmacovigilance Centre-in Turkey). Pharmacists encounter drug poisoning very infrequently (5.7%) and respond to it 100% of the time. In the most recent week of the study, no one applied for an HIV test. 94.1% of the pharmacists do not approve of the selling of over-the-counter drugs. As a result of this study, we learned that provisions for the primary health care services given at pharmacies were not present in existing legislation, although there is a demand for them in the affected communities.

Key words: pharmacist, primary health care, prescribed medicines, non-prescribed medicines.

INTRODUCTION

The concept of Primary Health Care Service first came to light in 1978 at a conference organized by the World Health Organization (WHO) and the United Nations International Children's Emergency Fund (UNICEF) in Kazakhistan's capital city Alma Ata.[1] According to the declaration made there, Primary Health

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Care Systems (PHCS) form the first link in a National Health Care System. The declaration stressed the importance of having access to a health care service chain as close as possible to people's homes and workplaces.[1] Pharmacies are by their very nature the first links in the health care chain. Health care services are divided into three groups: preventive medicine services, therapeutic health care services, and rehabilitation services. Preventive medicine services are also divided into two groups: outreach services and personalized services. Personalized services are carried out by healthcare professionals such as doctors, nurses and obstetricians.[2]

Because pharmacy services are included in personalized health care services, pharmacists should guide patients to consult doctor, and instruct them on how to use their drugs properly. Three sectors need to be considered with regard to health care services:

Folk sector. This sector is rejected by scientific medicine and official health care services. It is made up of people who are not officially educated or trained about health, but are the nonetheless considered an expert by society. Examples of people in this group are those who treat fracture-dislocation conditions, and obstetricians without diplomas.

Popular sector. This sector is made up of people who are not educated about health but considered as knowledgeable by people that they know. This group includes people like mothers, fathers, friends and elders.

Professional sector. This sector is made up of people who are formally educated and considered to be professionals by the scientific community. This group includes doctors, dentists, pharmacists, veterinarians, and nurses. Only a fraction of the health care services produced and consumed in societies are served by this sector.[3,4]

People often buy medicine from pharmacies after telling their complaints to the pharmacist on duty. and the purchase of medicine without prescription is quite common. This condition shows that pharmacies also serve as a part of the 'popular sector'. In Turkey, pharmacies are the most common health care unit used by the public as a primary health care service provider. Therefore, pharmacies play a key role in the use of prescribed medicine and in increasing consciousness about the proper use of prescribed medicine. The role of pharmacists in Primary Health Care Services has become a common topic in recent years in discussions between the WHO and the International Pharmaceutical Federation (FIP).[5] The role of pharmacies is better understood after a comparative analysis of health institutions, the number of pharmacies and applications to these places in both developed or developing countries; when evaluating data related to purchasing

unprescribed medicine, the role of pharmacies in these services is also better understood.[6,7] In 1980, the total number of pharmacies was 6.488, but by 1990, this number had reached 12.397, a 99% increase.[8] Today (2017) the number is 24.935 according to the provincial health directorate pharmacy license module. At the same time, the population per health clinic exceeded the population per pharmacy. Local research suggests that our people are prone to use medication without prescription, and, upon getting sick as a result, consult their doctor on their own initiative, or at a pharmacist's recommendation.[9,10] Patients either have these medications in their home already, or they purchase them from pharmacies.[11] Sometimes it is observed that expired prescriptions are repeated.[12,13] These medications can cause serious diseases. For example, a cold preparation that can be purchased without prescription can cause organic affective psychosis.[14] Additionally, it has been observed that commonly used antibiotics lose their effectiveness on bacteria over time.[15] It is reported that the acetylsalicylic acid in Non-Steroidal Anti-Inflammatory Drugs (NSAID), can cause esophagitis.[16] It is also a known fact that the unconscious use of various medications often produce more harm than expected therapeutic effect in patients.[7,17,18,19,20]

The duty of pharmacists in the face of these problems is to encourage and promote conscientous drug use by sharing general information about diseases and symptoms with doctors and other healthcare professionals.[5,6]

As an indispensable link in the healthcare institution chain, pharmacies should not only sell drugs to patients, but also raise people's awareness about them and, more importantly, prevent the use of inappropriate and unnecessary medications.

The aim of this study is to determine the purposes of the position of pharmacies in primary health care services chain, and to determine the services received from pharmacies, since these change according to the relative distances between pharmacies and health clinics.

METHODOLOGY

Time and Location of Research

This research was conducted between August 23rd, 2005 and September 12th, 2005 in Kırklareli (Turkey) a city with a population of 53,221.[21]

The number of pharmacies and pharmacists who completed the survey in Kırklareli province is given in Table 1. Kırklareli lies in the Marmara region of Turkey (Figure 1).

Table 1: The number of pharmacies and pharmacists who completed the survey in Kırklareli provinces.

City of Kırklareli	Number of Pharmacy	Number of Pharmacists Who Complete the Survey	Percentage (%)
Town Center	36	27	75.0
Babaeski	18	6	33.3
Lüleburgaz	44	14	31.8
Pınarhisar	4	2	50.0
Vize	5	2	40.0
Pehlivanköy	1	1	100.0
Demirköy	2	1	50.0
Beldeler	3	2	66.7
Total	113	55	

Figure 1: City map of Kırklareli

Data Collection and Evaluation

Surveys were distributed and collected by the Kırklareli Chamber of Pharmacists, and then given to the researcher. As a preliminary experiment, surveys were distributed to 44 pharmacies in İstanbul/Kadıköy and 23 pharmacists completed it (%52.27). According to a study of primary health care services by Gül *et al.*, surveys were distributed to a total of 73 pharmacies located on both the Anatolian and European sides of Istanbul. 40 of the 73 pharmacies completed the survey (%54.8).[22] According to another study by Gül *et al.*, surveys were distributed to 90 pharmacies in the İstanbul districts of Kadıköy and Fatih. This survey was completed by 73 pharmacists (%81).[23] The survey consists of 29 questions, 4 of which are open-ended. As indicated in the data encoding information given in the appendix, the data was closed, re-coded and entered to the SPSS 8.0 for Windows programme.

A Chi-squared test was used in the statistical evaluation of the research data.

Ethical Problems

We told pharmacists that neither their names nor the names of their pharmacies would be used in any kind of verbal or written explanation, and we have honored this commitment.

FINDINGS

Findings Related To Survey Data

Table 2: Distribution of Pharmacists by Number of Technicians Employed (Kırklareli, 2005)

Number of Technicians	n	%
0	1	1.9
1	28	52.8
2	20	37.7
3	4	7.6
Total*	53	100.0

*2 pharmacists did not answer this question.

Table 3: Distribution of Technicians by Working Hours (Kırklareli, 2005)

Working Time of Pharmacy Technicians (hour/day)	n	%
7	1	1.6
8	8	12.9
9	6	9.7
10	17	27.4
11	27	43.6
12	3	4.8
Total*	62	100.0

*14 pharmacists did not answer this question.

Table 4: Distribution of Technicians by Age (Kırklareli, 2005)

Age of Pharmacy Technicians	n	%
<19	8	11.8
20-29	41	60.3
30-39	8	11.8
40-49	5	7.3
50>	6	8.8
Total*	62	100.0

*9 pharmacists did not answer this question.

Table 5: Distribution of Technicians by Educational Status (Kırklareli, 2005)

Educational Status of Pharmacy Technicians	n	%
Primary School	16	20
Middle School	11	13.7
High School	38	47.5
University	15	18.8
Total*	80	100.0

*3 pharmacists did not answer this question.

Table 6: Distribution of Pharmacists by Gender (Kırklareli, 2005)

Gender of Pharmacist	n	%
Female	28	51.9
Male	26	48.1
Total*	54	100.0

*1 pharmacist did not answer this question.

Table 7: Distribution of Pharmacies Within 150 Meters of A Health Facility. (Kırklareli, 2005)

Is There A Health Clinic Near the 150 Meters of Pharmacy?	n	%
Yes	20	36.4
No	35	63.6
Total	55	100.0

Table 8: Distribution of Health Facilities Within 150 Meters of a Pharmacy (Kırklareli, 2005)

Health Facility Type That Near To 150 Meters of Pharmacies	n	%
Health Clinic	11	55.0
Public Hospital	7	35.0
Private Hospital	2	10.0
Private Policlinic	0	0.0
Public Policlinic	0	0.0
Total*	20	100.0

*35 pharmacists did not answer this question.

Table 9: Distribution of Prescribed Medications Sold in Pharmacies in the Most Recent Week (Kırklareli, 2005)

Prescripted Medication Groups That Sold in Pharmacies in the Most Recent Week	n	%
Analgesics	112	19.5
Antibiotics	74	12.9
Antirheumatic Drugs	65	11.3
Antihypertensives etc.	61	10.6
Heart Drugs	56	9.8
Vitamins	54	9.4
Antidepressants	34	5.9
Anti-Flu Medications	31	5.4
Lipid-Lowering Medications	24	4.2
Antihistamines	17	3.0
Hormones	15	2.6
Antiepileptics	12	2.1
Green Prescription Medicines	10	1.7
Aphrodisiacs	9	1.6
Total*	574	100.0

*11 pharmacists did not answer this question.

Table 10: Distribution of Non-Prescribed Medications Sold in Pharmacies in the Most Recent Week (Kırklareli, 2005)

Prescripted Medication Groups That Sold in Pharmacies in Last Week	n	%
Analgesics	42	24.1
Antibiotics	17	9.8
Antirheumatic Drugs	20	11.5
Antihypertensives etc.	9	5.2
Heart Drugs	8	4.6
Vitamins	22	12.6
Antidepressants	7	4.1
Anti-Flu Medications	15	8.6
Lipid-Lowering Medications	2	1.1
Antihistamines	16	9.2
Hormones	7	4.1
Antiepileptics	3	1.7
Aphrodisiacs	6	3.4
Total*	174	100.0

*15 pharmacists did not answer this question.

Table 11: Distribution of Herbal Products Sold in Pharmacies in the Most Recent Week (Kırklareli, 2005)

Packaged Herbal Product Groups That Sold in Pharmacies in Last Week		
	n	**%**
Weight Loss Preparations	7	63.6
Energizing Products	3	27.3
Dead Nettle Leaf	1	9.1
Sage Tea	0	0.0
Rosehip	0	0.0
Linden	0	0.0
Mint	0	0.0
Senna	0	0.0
Garlic	0	0.0
Total*	11	100.0

*37 pharmacists did not answer this question.

Table 12: Distribution of Patients Willing to Purchase Non-Prescribed Medications From Pharmacies (Gender and Age) (Kırklareli, 2005)

Age	**Gender**			
	Female		**Male**	
	n	**%**	**n**	**%**
<19	33	6.9	29	5.2
20-29	89	18.7	108	19.5
30-39	112	23.6	130	23.5
40-49	129	27.2	145	26.2
50>	112	23.6	142	25.6
Total*	475	100.0	554	100.0

*27 pharmacists did not answer this question.

Table 13: Distribution of Non-Medicinal Products Sold in Pharmacies in the Most Recent Week (Kırklareli, 2005)

Top-Selling Non-Medication Products in Last Week	**n**	**%**
Pregnancy Tests	27	20.8
Antiseptics e.g. tincture of iode	24	18.5
Plasters	21	16.2
Insecticides	15	11.5
Vitamins	15	11.5
Cosmetics	10	7.7
Baby Products	9	6.9
Family Planning Products	9	6.9
Slipper etc.	0	0.0
Toys	0	0.0
Total*	130	100.0

*15 pharmacists did not answer this question.

Table 14: Distribution of Healthcare Professionals Consulted First by Patients About Their Health Complaints (Kırklareli, 2005)

How Do Patients Report Health Complaints First?	**n**	**%**
To Pharmacist	26	48.1
To Doctor	28	51.9
Total*	54	100.0

*1 pharmacist did not answer this question.

Table 15: Reasons Why Patients First Consult Pharmacists (Kırklareli, 2005)

Reasons of Consulting Pharmacist First	**n**	**%**
Because it is free or because patients do not want to pay for both a doctor and medicine	15	45.45
Because access to a pharmacist is easier than access to a doctor	15	45.45
Because patients do not think that their sickness is serious	3	9.10
Total*	33	100.0

*22 pharmacists did not answer this question.

Table 16: Distribution of Warnings by Pharmacists That Patients Should Report Adverse Reactions and/or Side Effects (Kırklareli, 2005)

Are Warnings that the Drug Needs to be Reported to the Pharmacy If It Has Adverse Reactions and / or Side Effects Issued?	**n**	**%**
Yes	42	84.0
No	8	16.0
Total*	50	100.0

*5 pharmacists did not answer this question.

Table 17: Distribution of Healthcare Professionals Informed by Patients of Adverse Reactions and/or Side Effects (Kırklareli, 2005)

Which Healthcare Professionals are Informed when a Drug Has Adverse Reactions and/or Side Effects?	**n**	**%**
Pharmacist	30	57.7
Doctor	22	42.3
Total*	52	100.0

*3 pharmacists did not answer this question.

Table 18: Distribution of Pharmacist Response to Adverse Reactions and/or Side Effects (Kırklareli, 2005)

What is being done by pharmacists for those who complain of adverse reactions and/or side effects of drugs?		
	n	**%**
I guide the patient to a health facility	49	98.0
I intervene with the patient	1	2.0
I guide the patient to a private doctor	0	0.0
I call the pharmaceutical company	0	0.0
Total*	50	100.0

*5 pharmacists did not answer this question.

Table 19: Distribution of Applications to Pharmacists About Drug Poisoning (Kırklareli, 2005)

Are there any Applications About Drug Poisoning?		
	n	**%**
Yes	3	5.7
No	50	94.3
Total*	53	100.0

*2 pharmacists did not answer this question.

Table 20: Distribution of Pharmacist Response to Drug Poisoning (Kırklareli, 2005)

What is being done by pharmacists for those who encounter drug poisoning?	n	%
I guide the patient to a health facility	3	100.0
I intervene with the patient	0	0.0
I guide the patient to a private doctor	0	0.0
Other	0	0.0
Total*	3	100.0

*52 pharmacists did not answer this question.

Table 21: Distribution of Non-Drug-Requests by Patients in the Most Recent Week (Kırklareli, 2005)

Services	n	%
Drug counseling	23	82.1
Preparation of Solution/Drug	4	14.3
Bandages	1	3.6
Total*	28	100.0

*26 pharmacists did not answer this question.

Table 22: Distribution of Consultation with Pharmacy for HIV Tests in the Most Recent Week (Kırklareli, 2005)

Has any kind of help been requested regarding HIV tests in the past week?	n	%
Yes	0	0.0
No	51	100.0
Total*	51	100.0

*4 pharmacists did not answer this question.

Table 23: Distribution of Terminating Drug Supplies in the Most Recent Week (Kırklareli, 2005)

Drug Supplies for Terminating Pregnancy	n	%
Yes	7	13.7
No	44	86.3
Total*	51	100.0

*4 pharmacists did not answer this question.

Table 24: Distribution of Pharmacists Responses to Patients Wishing to Terminate Pregnancy (Kırklareli, 2005)

How are Patients guided by Pharmacists About Terminating Pregnancy?	n	%
I guide the patient to a doctor	3	42.9
In the first 12 hours I suggest estrogen at 100 mg a day, after 72 hours, I suggest another 100 mg of estrogen	2	28.5
I guide the patient to a health facility	1	14.3
I do not give any service	1	14.3
Total*	7	100.0

*49 pharmacists did not answer this question.

Table 25: Distribution of Family Planning Methods Purchased in the Most Recent Week (Kırklareli, 2005)

Family Planning Methods	n	%
Oral contraceptives	17	43.5
Male condoms	15	38.5
Suppository, foam, gel	5	12.8
Female condoms	1	2.6
Intrauterine devices	1	2.6
Diaphragma	0	0.0
Total*	39	100.0

*12 pharmacists did not answer this question.

Table 26: Distribution of Approvals by Pharmacists About the Selling of Over-The-Counter Drugs Outside of Pharmacies (Kırklareli, 2005)

Do pharmacists approve the selling of over-the-counter drugs outside of pharmacies?	n	%
Yes	2	5.9
No	32	94.1
Total*	34	100.0

*21 pharmacists did not answer this question.

Table 27: First Application Place to a Pharmacy Within 150 Meters of a Health Facility (Kırklareli, 2005)

Is there a health facility within 150 meters of the pharmacy?	First Professional Sought in Complaints				Total	
	Firstly Doctor		Firstly Pharmacist			
	n	%	n	%	n	%
Yes	7	36.8	12	63.2	19	100
No	19	54.3	16	45.7	35	100
Total	26	48.1	28	51.9	54	100

$X^2=1.501$ $p=0.221$

Table 28: Distribution of Consultatitons for Terminating Pregnancy According to the Gender of the Pharmacist (Kırklareli, 2005)

Gender of Pharmacist	Requesting Help for Terminating Pregnancy			
	Total		Yes	
	n	%	n	%
Male	26	52	2	28.6
Female	24	48	5	71.4
Total	50	100	7	100

fisher exact test=0.239

DISCUSSION AND CONCLUSION

This research was conducted between August 23rd, 2005 and September 12th, 2005 in Kırklareli. 55 of the 113 pharmacists who registered with the Chamber of Pharmacists (Table 1). According to a study of primary health care services by Gül *et al.*, surveys were distributed to 73 pharmacies located on both Anatolian and European sides of İstanbul. And 40 pharmacies completed the survey (%54.8).[22] According to another study of primary health care services by Gül and his associates, surveys were distributed to 90 pharmacies in the Kadıköy and Fatih districts of İstanbul. That survey was completed by 73 pharmacists (%81).[23] The survey consists of 29 questions, 4 of which are open-ended. As indicated in the data encoding information in the appendix, the data was closed, re-coded and entered to the SPSS 8.0 for Windows programme.

In this study, we learn that 52.8% of pharmacists have only one technician as indicated in Table 2. Additionally, the ratio of pharmacists working with 2 technicians was 37.7%. Gürses and Aran *et al.* also shows that pharmacists work with one or two technicians.[24] The pharmacists in our research region state that one or two technicians are sufficient for general pharmacy works, considering the population. In Table 3, percentage of pharmacists whose technicians work 7, 8, 10, 11 and 12 hours a day were determined as 1.6%, 12.9%, 27.4%, 43.6%, and 4.8%, respectively.

Table 4 indicates that the age range of technicians is mostly between 20-29. Gürses and Aran *et al.* stated that the age range of technicians is primarily 25-34.[24] Since the technicians are relatively young, 43.5% of them work 11 hours a day. In Table 5, percentage of technicians at with a high school diploma or higher is 66.3%. All of technicians who are university graduates are close relatives of pharmacists. According to a study conducted by Boztok *et al.* in İzmir, 46.7% of technicians held a high school diploma or higher.[25] The gender of the pharmacists who participated in this study are shown in Table 6; the percentage of female pharmacists is 51.9% while ratio of male pharmacists was 48.1%. Thus there is an equal gender distribution with regard to gender. Table 7 shows the

distribution of pharmacies that are within 150 meters of a health facility. The percentage of pharmacies with no health facility within 150 meters is 63.6%.

Table 8 indicates that 55% of health facilities within 150 meters of pharmacies are health clinics. According to Boztok, 48.7% of health facilities in closest proximity to a pharmacy are health clinics.[25] Thus it can be seen that health clinics are the most common health facilities and patients can easily reach a pharmacy after leaving a health clinic. Table 9 illustrates that the highest rate of patients visited pharmacies to purchase prescribed-analgesics (19.55%) in the most recent week of the study. The products with the lowest rate of purchase were aphrodisiacs (1.6%).

Table 10 shows that the top-selling products without a prescription were analgesics, vitamins and antirheumatic drugs. Generally, antibiotics are used for upper respiratory tract infections like tonsillitis, pharyngitis and laryngitis. There is a decrease in the sale of heart medicines and antihypertensives because elderly patients generally go to areas with warmer climates in the Summer season. According to Boztok, 35.6% of all medicines sold in pharmacies are analgesics and antirheumatic drugs.[25] According to another study by Hannay, the most commonly used non-prescription drugs are antipyretics, analgesics, antitussive drugs and laxatives, respectively.[26] According to Vicencio *et al.*, the drugs that are sold most are analgesics, NSAID's and vitamins.[27] In Table 9 sales of aphrodisiacs occur at a rate of 1.6%, whereas in Table 10, sales of aphrodisiacs occur at a rate of 3.4%, an indication that more aphrodisiacs are being sold.

The excessive sale of weight-loss and energy products is attributed to excessive advertising for these products (Table 11). The majority of male patients who want to buy medicines without prescriptions are over the age 40. There is not a significant difference between males and females by gender in the frequency of medicine purchase (Table 12). It is reported that pregnancy test products are the most popular non-medicinal products with a rate of %20.6 in the most recent week of the study. The least-sold products are toys, slippers etc. (Table 13).

Patients apply first to doctors and then to pharmacists with rates of %51.9 and %48.1 respectively with their complaints (Table 14). In a study conducted in Ankara by Onaran, patients applied first to pharmacists for their complaints at a rate of %43.8.[28] We think that pharmacies play a key role in this situation because they are well-educated and easily accessible. When evaluating the reasons for applications to pharmacists by patients, the fact that pharmacies are free and easily reachable, and those patients' complaints are not significant are the reasons not to visit a doctor (Table 15).

According to Table 16, 84% of pharmacists warn patients that they should report

adverse reactions and/or side effects of medications when they occur, and 57.7% of patients report adverse reactions and/or side effects to pharmacists first (Table 17). According to a study conducted by Uğraşbul and Sütaş in İzmit, 62.1% of patients report the adverse reactions and/or side effects to pharmacists first. 92.2% of pharmacists guided such patients to a health facility. Table 18 shows that 98% of pharmacists guided patients who report adverse reactions and/or side effects to a health facility. Pharmacists also report the adverse reactions and/or side effects of these medications to TÜFAM (The National Pharmacovigilance Centre in Turkey).

According to our study, 94.3% of pharmacists report that there are no applications for drug poisoning (Table 19). All of the pharmacists guided patients with drug poisoning to a health facility (Table 20). We think that patients apply to pharmacies first because they are more common than health facilities.

According to Table 21, the percentage of patients who come to a pharmacy for drug counseling 82.1%. According to another study, this percentage is 28.8%.[24] It can be seen that patients mostly get help from pharmacies for purchasing and counseling about medicine.

Our study shows that the number of patients who come to the pharmacy for counseling about HIV tests is very low. It can be thought that reason for this situation is patients' concerns over privacy or embarrassment (Table 22). The reason for our HIV test research is that Kırklareli is a borderline province and HIV tests are sold in pharmacies.

Oral contraceptives are given to 13.7% of patients who want to terminate pregnancy by 45.5% of pharmacists, acccording to Tables 23 and 25. When evaluating the question in Table 24 (to which only 7 pharmacists responded) it is seen that 42.9% of pharmacists guided patients to a doctor willing to help in terminating pregnancy. In another study conducted by Uğraşbul and Sütaş in İzmit, 32.6% of pharmacists sold patients the necessary medicine, whereas 27.2% of pharmacists guided patients to a health facility.[29] Our study shows that the most preferred methods for family planning are oral contraceptives and male condoms at rates of 43.5% and 38.5%, respectively. Oral contraceptives can also be used for treating acne vulgaris and as a hormone replacement treatment (Table 25). According to a study conducted by Boztok in İzmir, male condoms and oral contraceptives are the most preferred method for family planning at rates of 40.7% and 38.9%, respectively.[25] It can be seen that the most preferred methods are the safest methods.

94.1% of pharmacists oppose selling over-the-counter medicines outside of pharmacies (Table 26). In a study conducted by Boztok, 97.6% of pharmacists

oppose the selling of over-the-counter medicines outside of pharmacies, and state that these kinds of medicines need to be sold in pharmacies.[25] McFadyen *et al.* reported that the incorrect use of over-the-counter medicines like insomnia pills, codeine, drugs that contain caffeine or pseudoephedrine, antitussive mixtures, laxatives and especially some analgesics is common.[30] Deshpande and Tiwari show that 31% of patients use over-the-counter medicines. 26.9% of such patients are between the ages of 31-40, and 30.8% are in the 41-50 age range. The use of these medications is more common in men than in women.[50]

Table 27 shows that pharmacists were applied to first by patients when drug side effects occurred 51.9% of the time. 63.2% of these pharmacists state that a health facility exists within 150 meters of their pharmacy. The location of the first application differs depending on whether there is a health facility within 50 meters of the pharmacy.

There is no significant statistical difference between genders of pharmacists about requesting help for terminating pregnancy (Table 28). A small number of patients prefer female pharmacists to male pharmacists when seeking help in terminating pregnancy.

When evaluating the open-ended question that asks how cold-chain vaccine application is performed, it can be seen that pharmacists pay attention to storage conditions and sale style. It is understood that pharmacists bring cold environment vaccines from a pharmaceutical warehouse, store them in a fridge, and sell them patients with ice or an ice gauge. Most of applications by patients to pharmacists are about conditions like diarrhea, pain, cold and the use of medicines.

It is thought that the most effective way to counter non-prescription drug use is state policy and community education. When investigating how patients behave when they get sick, it is seen that first, they try to cure themselves, and if this doesn't work, they apply to the nearest health facility, which is generally a pharmacy. Finally, if patients think that their condition is serious, they apply to a doctor. It is thought that the best way to solve this problem is to make people adopt the correct treatment methods by raising public awareness.

Suggestions That Can Be Offered As A Result of This Study

– All doctors and pharmacists should be encouraged to attend at least one symposium about medication and its developments, regardless of whether they are private or public sector workers.

– Authority sharing of all health care professionals should be determined by comprehensive research, and all health care professionals should be encouraged to comply with these results.

– Use of non-prescription medication should definitely be prevented. A list of medications that can be sold without prescription should be compiled and results should be announced to doctors and pharmacists by the health authorities.

– The public should be provided with information about the incorrect use of medication through publishing campaign programmes in newspapers, magazines, television and radio.

– Scientific research should be made about the reasons for the use of medication without a prescription.

– The public should be encouraged to use prescription medication by making health insurance more common.

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