AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

A Novel Structured Emulsion Containing Oleocanthal Enriched Extra Virgin Olive oil and Curcumin

Koteswari P[1]*, Gowthami P[2], Deepika Y[2], Abhishek Kumar G[2]
1.Department of Pharmaceutics, Srimathi Sarojini Ramulamma college of pharmacy, Mahbubnagar, Telangana, India
2.Department of Pharmaceutics, Hindu College Of Pharmacy, Guntur, Andhra Pradesh, India – 522007.

ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune destructive joint disease that is caused by inflammation in the tissue that normally produces lubrication fluid for joints. Till now there is no known cure for RA and the goal of treatment is to reduce joint inflammation and pain. The present investigation is to develop a novel formulation called structured emulsion using extra virgin olive oil (EVOO) enriched with oleocanthal and curcumin. Oleocanthal and curcumin are recently recognized for their anti-rheumatoid and anti-cancer properties. The primary objective of this investigation is preparation of EVOO extract containing oleocanthal as major fraction. The second objective is inducting this oleocanthal fraction into EVOO to enrich EVOO with oleocanthal and this is taken as oil phase. The structured emulsion was prepared and evaluated for various physicochemical properties such as viscosity, spreadability, extrudability, pH, Curcumin content. FTIR studies were carried out to identify any interactions between curcumin ,oleocanthal and other excipients used in the formulation. Ex-vivo studies were conducted for determining the curcumin release rate. The emulsion exhibited pseudoplastic behavior and thixotropic properties. The ex vivo studies showed first order drug release. FTIR studies revealed that there was no drug or drug excipient interactions. Curcumin loaded oleocanthal enriched structured emulsion was prepared successfully and evaluated.

Keywords: oeocanthal, curcumin, structured emulsion

*Corresponding Author Email: polurikoteswari@gmail.com
Received 02 October 2019, Accepted 17 October 2019

Please cite this article as: Koteswari *et al.*, A Novel Structured Emulsion Containing Oleocanthal Enriched Extra Virgin Olive oil and Curcumin. American Journal of PharmTech Research 2019.

INTRODUCTION

In Mediterranean countries, since ancient biblical times, olives as fruits and olive oil as primary cooking fat obtained from Olea europaea are consumed by people. The health benefits of olive oil have been explored enormously in recent years (1&2). Its high oleic acid content makes it less susceptible to oxidation and therefore it has high stability and long shelf life.

Oleocanthal is a substance derived from extra virgin olive oil and chemically it is described as 2-(4-hydroxyphenyl) ethyl ((3S,$E)-4-formyl – 3 – (2-oxorthyl)hex-4-enoate.Oleocanthal is also known as deacetoxy ligstroside aglycon. Existing evidence also described about the other pharmacological activities such as antioxidant, anti-inflammatory, neuroprotective and anti cancer properties (3&4). In one study it is reported that oleocanthal kills most types of cancer cells within half an hour. Oleocanthal is also reported to be effective in the treatment of cognitive disorders like Alzheimer's. However, the pharmacokinetics of oleocanthal is not reported so far in animal models or humans. Another recently explored bioactive is curcumin, a diary heptanoid reported to be effective in rheumatoid arthritis, Alzheimer's and various types of cancers. Although it is also effective against many types of cancers and rheumatoid arthritis, its poor stability, photosensitivity and poor bioavailability hindered its use pharmacologically.

Rheumatoid arthritis is an autoimmune disease affecting more than two million people all over the world. Several NSAIDs can be used to treat RA, but they can cause gastric irritation, renal failure and complete cure is not possible. Recently, with the advent of biotechnology, various biologics such as cimizia, enbrel, rituxin and so on, have been approved to treat RA. However these biologics must be administered by self injection or by IV infusion. This method of treatment is expensive and even not covered by insurance. Because of these reasons the researchers are looking to develop novel drugs and drug delivery systems. Hence it is hypothesized that when these compounds, are administered concurrently will produce an additive effective in both rheumatoid arthritis and many types of cancers. Till date no formulation containing these compounds either for topical or oral administration was reported in the literature. Therefore the objectives of the present investigation were to extract oleocanthal from EVOO (extra virigin olive oil) and to develop a novel topical emulsion formulation containing oleocanthal enriched olive oil and curcumin for systemic use with multiple pharmacological benefits.

MATERIALS AND METHOD

Extra Virgin Olive oil was purchased from Bragg live food products, Box 7, Santa Barbara, CA93102, and United States. Curcumin was purchased from Laila Nutraceuticals, Auto Nagar,

Vijayawada, Andhra Pradesh 520007, India. White soft paraffin, Sorbitol mono stearate, Hydroxy propyl methyl cellulose, Methyl paraben, propyl paraben, Polysorbate – 80, Tocopherol, and emulsifying wax were all procured from the Department of Pharmaceutics, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India.

Extraction of oleocanthal from olive oil

Oleocanthal from olive oil was extracted by liquid extraction procedure. A weighed quantity of olive oil was dissolved in cyclohexane. Acetonitrile was used as extracting solvent. A portion of acetonitrile was added to the oil and cyclohexane solution. The entire mixture was kept on an orbital shaker for 15 min at 25°C. After the completion of shaking the mixture was centrifuged at 4000 rpm for 5 min. The centrifuged mixture forms two separate phases and the acetonitrile phase was collected and kept aside. The procedure was repeated with second and third portions of acetonitrile. All the collected fractions were pooled and acetonitrile was evaporated using rotavac for at least 1 hour at 45°C and at 50 rpm. The oil extract containing oleocanthal was packed in an amber colored bottle and stored in a dark place until it is used (5).

Formulation development and preparation of structured emulsion

The structured emulsion for topical application was prepared by homogenization. To prepare oil phase a weighed amount of EVOO was taken into a 50ml beaker. Then accurately weighed quantities of olive oil extract and curcumin were added to the EVOO. Required amounts of white soft paraffin, sorbitol monostearate, emulsifying wax were added to the above mixture and heated on a water bath at 70°C for 15 min. Then propyl paraben and tocopherol were added and the whole contents were transferred into a mortar and triturated slowly in unidirectional until it forms a smooth mixture. To prepare aqueous phase, water was divided into portions. To the first portion, tween 80 and methyl paraben were added and dissolved. This portion was slowly added to the oil phase while triturating to yield an o/w emulsion. Then hydroxypropyl methyl cellulose E50 (HPMCE50) was added to the second portion of water and dissolved. This solution was added to the emulsion prepared and trituration continued for 5 min. Later the emulsion was transferred into another beaker and homogenized for 15 min at 5000 rpm. Then the formulation was transferred into an amber colored glass container, closed and stored in dark cool place until it is evaluated. Various formulation compositions were given in the **table 1**

Table 1 Compositions of topical gel

Composition/Code	F1	F2	F3	F4	F5	F6	F7
EVOO extract	-	-	-	-	-	-	10mg
Curcumin	-	-	-	-	-	-	10mg
*EVOO (gms)	30%	30%	30%	40%	40%	30%	40%

White soft paraffin	5%	12%	5%	8%	5%	5%	5%
**Span 60	12%	12%	12%	12%	12%	12%	14%
HPMC E 50	-	-	0.1%	0.5%	5%	2.5%	5%
Methyl paraben	-	-	-	0.15%	0.15%	0.15%	0.15%
Propyl paraben	-	-	-	0.15%	0.15%	0.15%	0.15%
Tocopherol	-	-	-	-	1%	1%	1%
Tween-80	2%	2%	2%	2%	0.15%	0.15%	0.15%
Emulsifying wax	4%	4%	4%	6%	-	-	-
Distilled water	10ml	10ml	10ml	10ml	10ml	10ml	10ml

***EVOO – extra virgin olive oil**
****Span 60 - Sorbitan monostearate 60**

Measurement of pH

The pH of the emulsion was measured using a digital pH meter. The glass electrode was completely dipped into emulsion system to cover the electrode and the pH was recorded. The measurements were made in triplicate and the average of the three readings was recorded.

Determination of rheological properties

Brookfield viscometer (LVDV-II+PRO) with spindle CPE-42 was employed while keeping the temperature constant during the study of rheological properties. Spindle was fixed firmly onto the shaft of viscometer with spanner. A quantity of sample was placed in cup and replaced the cup without hitting the spindle. The viscometer was operated in standalone mode at different rpm. To construct the rheogram the speed of the spindle was slowly increased from 1 rpm to 5 rpm to obtain the up curve and then decreased to obtain the down curve. The viscosity, % torque, shear stress and shear rate were noted. The rheograms were constructed and determined the type of flow and viscosity.

Fourier transform infrared spectrophotometer (FTIR)

FTIR Spectrometry was found to be most reliable technique for predicting the possible interaction between the drug and excipients. The spectra were recorded for curcumin and for olive oil extract and optimized composition of emulsion using FTIR (bruker), model: Alpha, TAT-001 opus software. The samples were scanned from 1000 to 3500 cm^{-1} and the obtained spectra were evaluated for drug excipient interactions.

In-vitro permeation studies using egg membrane as bio membrane

The contents of egg were removed by making hole on egg. Then the shell was dipped into HCl acid to dissolve the shell leaving membrane. The membrane was washed in water and used for in vitro permeation studies. (6).

Assembling of franz-diffusion cell

The membranes were then placed between the donor and receptor compartments of the cells, in

direct contact with the receptor medium. Approximately 150ml of the phosphate buffer pH6.4 was placed in the receptor compartment. Its temperature was maintained at 37±5°c using a thermostatic water bath. This whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was continuously stirred during the whole experiment using magnetic bead. Aliquot samples of 0.5 ml were withdrawn at predetermined time intervals and an equal volume of diffusion medium was replaced each time. Absorbance of the samples was measured spectrophotometrically at 419nm taking phosphate buffer solution pH 6.4 as blank. The amount of drug permeated per square centimeter at each time interval was calculated and plotted against time

RESULTS AND DISCUSSION

EVOO was evaluated for saponification value, acid value and iodine value and determined as 201.6, 66.198 and 90.73 respectively. The pH, spreadability and Extrudability of the selected formulation F7 were found as 8.6±0.7, 14.2±0.6 and 16±0.7 respectively. The Pseudoplastic viscosity of the gel was determined from the slope of tangent drawn to the shear stress vs shear rate curve and calculated as 1.09 centi poise. The plastic flow behavior and thixotropic properties of the emulsion were given in figures 1&2 and table 2, the FTIR spectral reports were shown in figures 3,4 and 5.

Ex vivo drug release studies were carried out for pure drug curcumin and the formulation and the results were depicted in figure 6,7 and 8. Oleocanthal was not quantified due to the unavailability of analytical method.

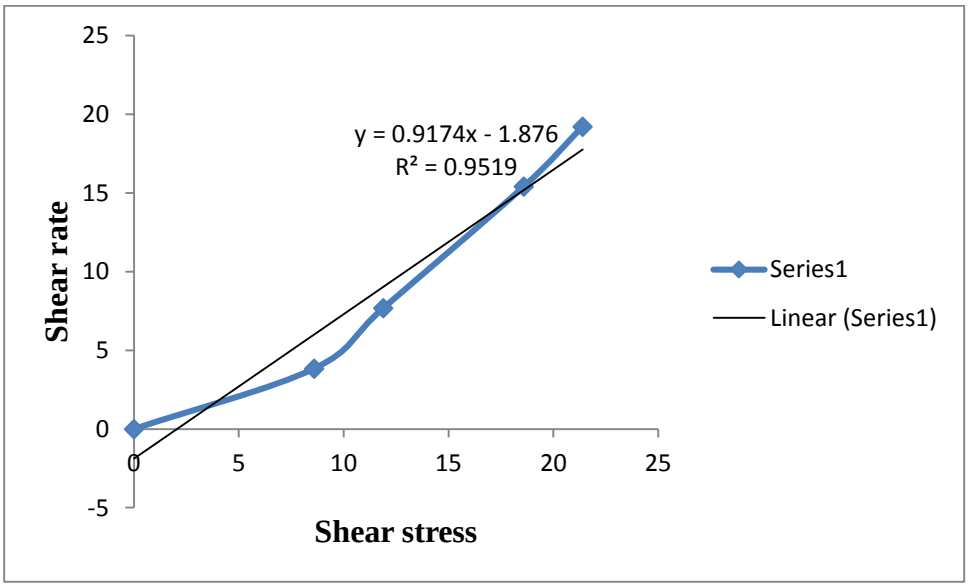

Figure 1: Rheogram representing pseudoplastic flow

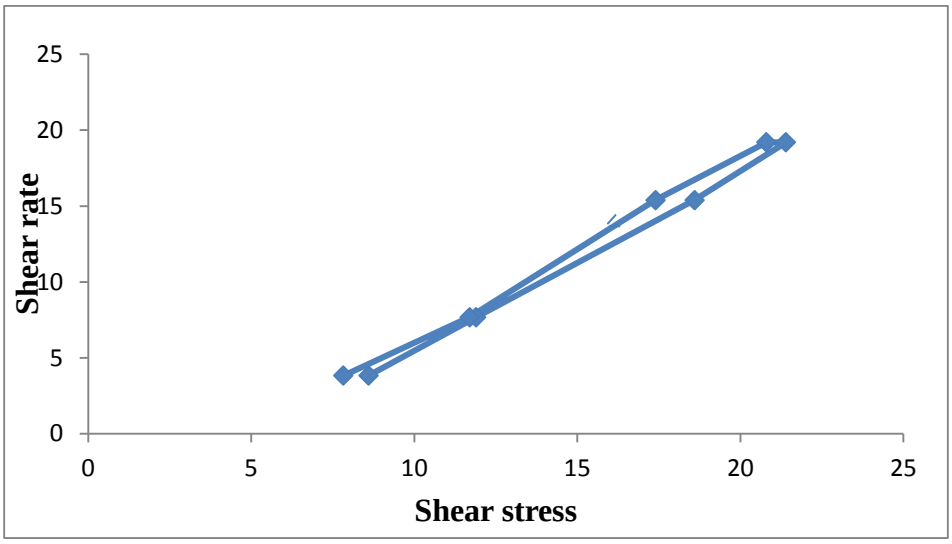

Figure 2 - Rheogram showing thixotropic behavior

Table 2 Rheological properties of topical gel F7

Viscosity	Speed	%Torque	Shear stress D/cm^2	Shear rate 1/sec	Temperature℃
219	1	36.9	8.6	3.84	29.7
162.3	2	52.5	11.9	7.68	29.6
122.7	4	80.3	18.6	15.4	29.6
109.2	5	92.3	21.4	19.2	29.5
109	5	89.5	20.8	19.2	29.5
115.9	4	75.5	17.4	15.4	2.6
149	2	50.6	11.7	7.68	29.6
208.8	1	34.4	7.83	3.84	29.7

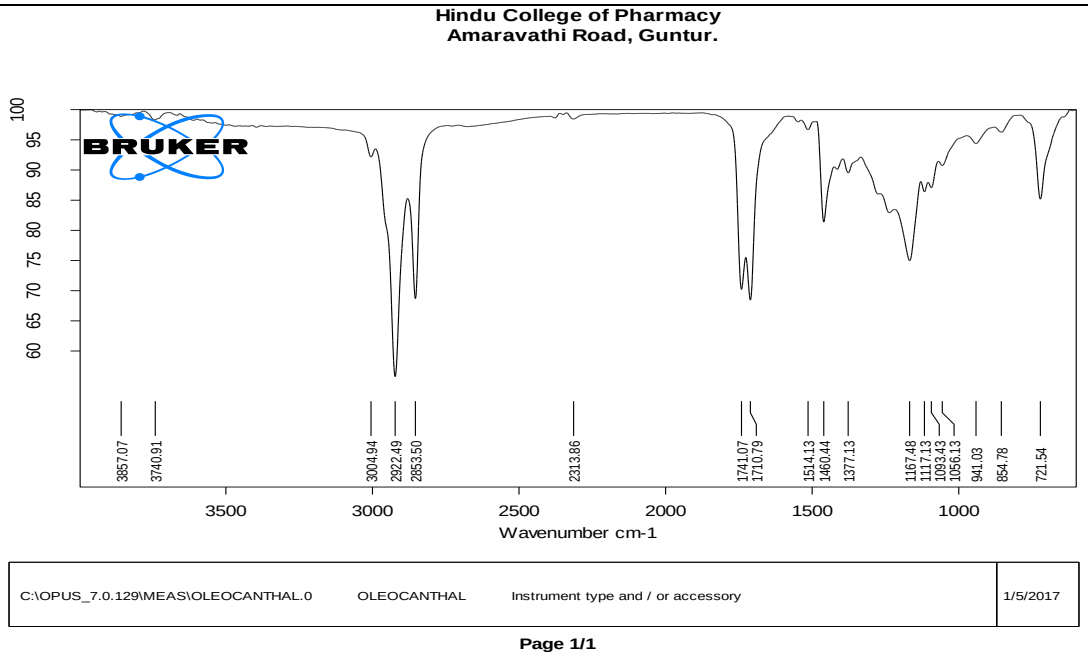

Figure 3: FTIR spectrum of EVOO extract representing oleocanthal

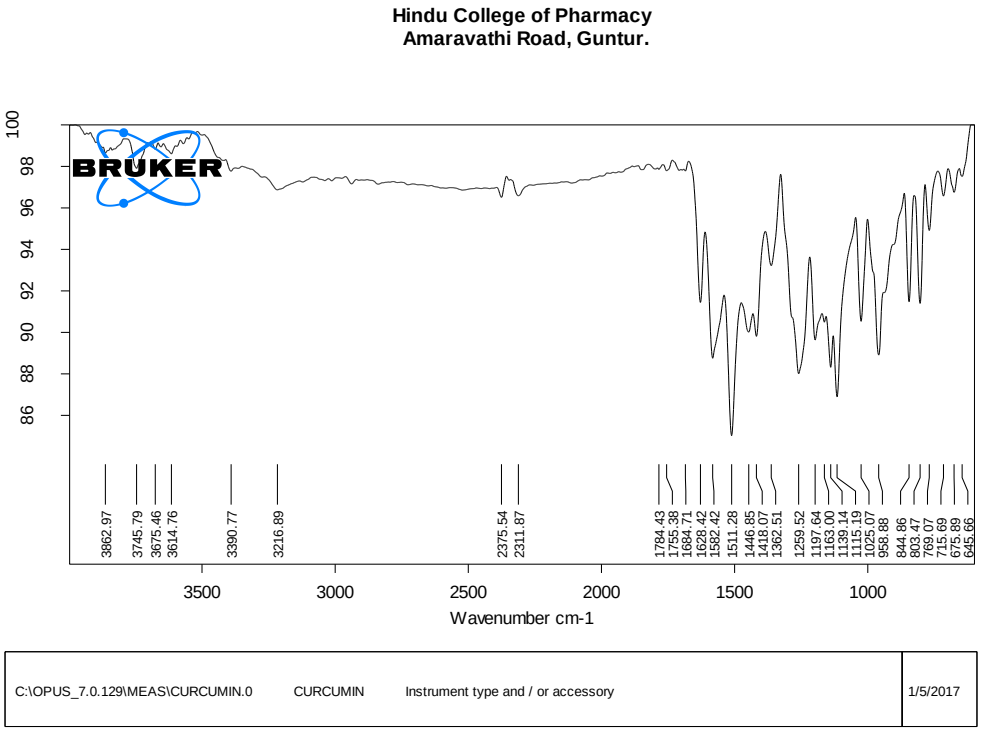

Figure 4: FTIR spectrum of curcumin

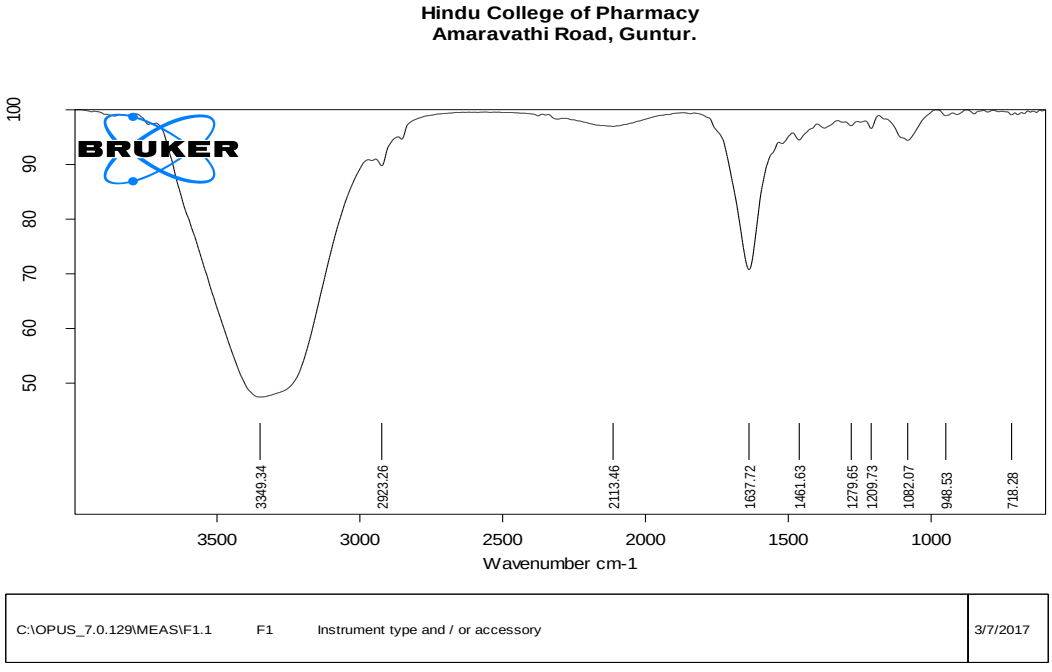

Figure 5: FTIR spectrum of optimized formulation

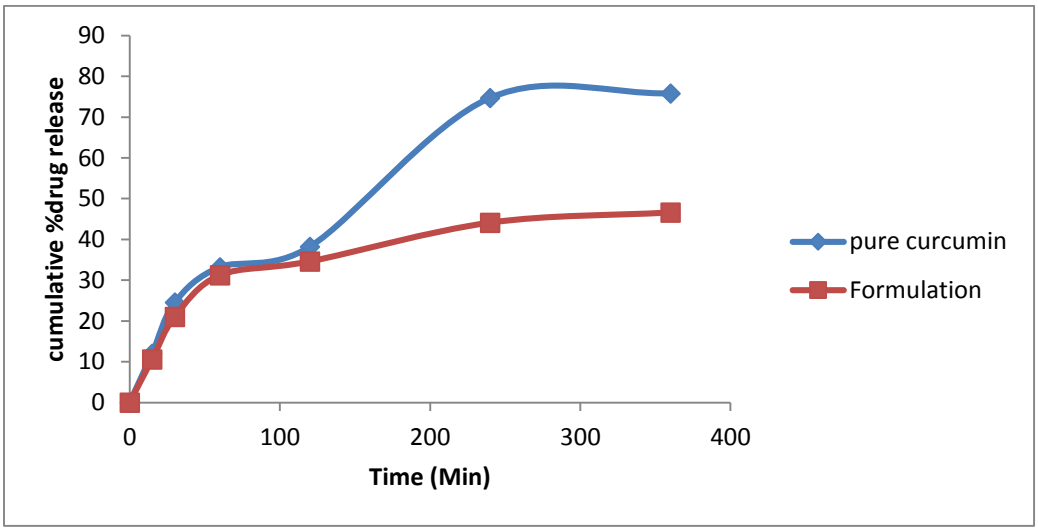

Figure 6: Cumulative % drug released in phosphate buffer pH 6.4

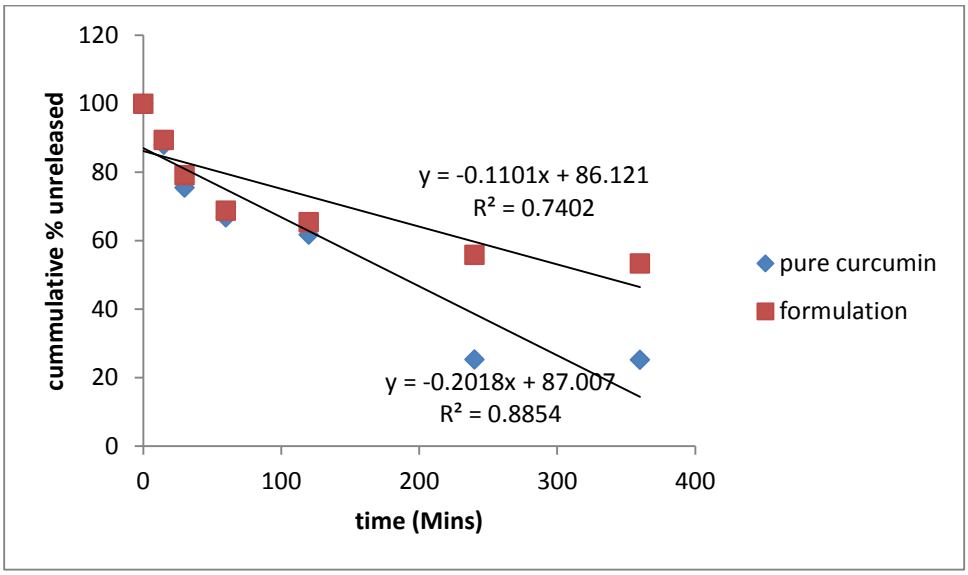

Figure 7: Percent drug unreleased in phosphate buffer pH 6.

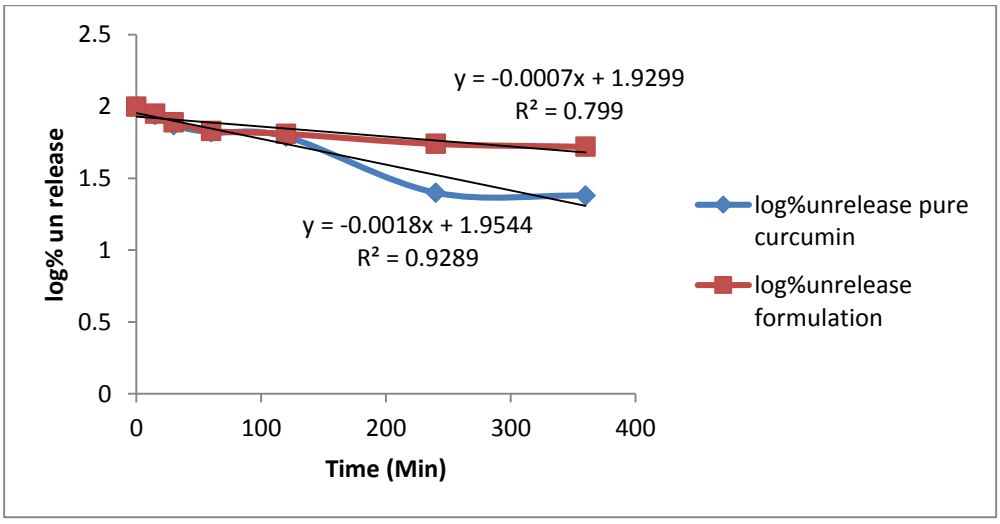

Figure 8: Log % drug Unreleased in phosphate buffer pH 6.4 after 6 hrs

DISCUSSION

In this investigation EVOO was used as main lipid phase and hence its saponification value, iodine value and acid value were determined. Saponification value and iodine value are important parameters to determine the quality of oils, because they give the chemical nature of oil. High saponification value is an indication for the presence of high proportion of shorter carbon chain lengths of the fatty acids. In combination with acid values, saponification values provide information regarding quantity and type of glycerides and the average weight of the acid in a given sample. Iodine value gives the degree of unsaturation in fats and oils. Iodine value also reflects the susceptibility of oil to oxidation. According to the Codex Alimentarius, International food standards, the saponification value of virgin and refined olive oils is in the range of 184-196 and that of iodine value is 75-94. The saponification value and iodine value of EVOO selected for the present investigation was found to be 201.6 and 90.73 respectively. Therefore, it is concluded that the oil used for the present investigation was in accordance with the AICC standards (7&8).

Oleocanthal from EVOO was extracted using the method described by Evangelia et al. Acetonitrile was used as extraction solvent because acetonitrile was not reactive with oleocanthal and one extraction with acetonitrile was sufficient to recover 85% of oleocanthal (9). In this investigation a hot process was used to produce an o/w emulsion. Extra virgin olive oil and white soft paraffin constituted the oil phase. Span 60 is attributed as emulsifying agent and also for structuring the oil. Propyl paraben and methyl paraben were incorporated as preservatives. Tween 80 was added to the formulation to enhance the physical stability and permeability characteristics of the formulation. HPMC was included to impart viscosity to the formulation. The EVOO extract contains oleocanthal and oleocin as major components and oleocanthal as the major fraction. It is difficult to separate oleocanthal and oleocin as these two compounds exist as diastereomers which are inseparable by conventional column chromatography. Hence in the present investigation oil extract was added to olive oil to enrich the oil with oleocanthal. The reason oleocanthal is available as micro constituent in EVOO and its efficacy depends on dose. Therefore, and as it is difficult to separate oleocanthal from its diastereomer extract was directly added to obtain better results when applied clinically.

The pH of the formulation was 8.6±0.7 units. This pH was just above the normal skin pH range and expected that it will not cause any skin irritation. Though, oleocanthal, curcumin are phenolic compounds which are slightly acidic in nature this pH may be due to the added materials such as emulsifying wax or the surfactants and the lipid phase. Extrudability test was based on the amount of formulation extruded from the collapsible tube upon application of weight. More the quantity of emulsion extruded better will be the extrudability. The formulation has good extrudable properties. The spreadability is very much important as it represents the behavior of emulsion when it comes

out from the device/tube. The spreadability was found to be 2.401g.cm/s and 19.44g.cm/s. The results indicated that the formulation was easily dispensable with a little shear stress (10).

The rheological properties of formula F7 were clearly indicated from the rheograms. The rheogram 1A showed the pseudoplastic behavior of emulsion in which the curve possess through the origin and when the shear stress was reached to a point the rate of shear was increased linearly. There was no yield value. The pseudoplstic viscosity was calculated from the tangent. The rheogram 1B depicted the thixotropic behavior that is shear thinning behavior of the formulation. With increasing rate of shear the shear stress increased and after reaching to a point the rate of shear stress was decreased and the down curve shifted towards the left making a hysteresis loop. The area of hysteresis loop was low and scissoring occurred when the shear rate was just below 10 rpm. This scissoring may be due to the reformation of structure of emulsion. Low viscosity and thixotropic behavior of formulation were important properties for uniform spreading of the topical preparations in fact viscosity and spreadability are inter related properties of topical formulations (11). The rheograms were showed in **figures 1 & 2.**

FTIR

The strong and intense band at 1684.71cm^{-1} is an indication of C═O stretch and is a characteristic functional group of curcumin. The strong peak at 1628 cm^{-1} is attributed to the C ═ C and at 1511 corresponds to C – O.

In oleocanthal FTIR spectrum, strong intense peaks at 2911.49 and 2853.5 cm^{-1} represents the presence of C SP^3 – H and C SP^2 – H vibrations. A band at 3004 cm^{-1} is an indication of un saturation and aromatic rings. Sharp absorption bands at 1741 cm^{-1} were attributed to ketone.

Hydroxyl function is one of the important characteristics of all infrared frequencies. In most chemical environments hydroxyl group does not exist in isolation, in fact a high degree of association is present as a result of hydrogen bonding with other hydroxyl groups. This hydrogen bonding will cause a significant band broadening and lower the mean absorption frequency. Compounds which exhibit extremely strong hydrogen bonding, a large shift to lower frequencies is observed due to the formation of a stable dimeric structure. However, the IR spectra of pure drug curcumin or olive oil extract containing oleocanthal did not show any narrow broad peaks in the region of 3500 to 3640 cm^{-1}. But characteristic narrow bands were observed in spectra of curcumin, oleocanthal and formulation in the region 1418.07 cm^{-1} - 1362.51 cm^{-1}, 1377.13 cm^{-1} and 1461.63 and 1279.65 cm^{-1} respectively. This absorption is important for certain hindered phenolic antioxidants. Because when phenolic group is isolated as a result of stearic hindrance or the sample is present in the vapor state or the sample is in dilute non polar solvent a characteristic narrow band is produced at the natural high frequency. Therefore it is concluded that being oleocanthal and curcumin are natural phenolic compounds and obtained from functional foods,

their IR spectra sowed narrow and characteristic peaks at high frequency region. Another important feature of oleocanthal is the presence of c-o-c, in which hydrogen is replaced by -CH=O. Bonding on both sides of oxygen results in ester bonds and IR spectroscopy is sensitive in identifying the ester functions. Therefore, a characteristic peak was observed at 1093.43 cm^{-1}, 1083 cm^{-1} in oleocanthal and formulation respectively and the approximate range is between (1150 and 1050 cm^{-1}).

In formulation IR spectrum a broad and intense band was observed at 3349.34 cm-1. This is considered as overtone or combination band which was resulted from mixed vibrations of different O – H stretches present in the formulation excipients. C – SP3 – H stretch and C – SP2 – H that are characteristic for oleocanthal were present in IR spectrum of oleocanthal and formulation. In formulation C ═ O was shifted to 1637 as broad band which is also a combination band of C ═ O, C – O and C ═ C (12).

From the FTIR studies it is revealed that all the functional groups present both in curcumin and oleocanthal are also present in the formulation and therefore it is concluded that there is no functional bond deformation and hence the formulation is stable and no interactions.

In vitro permeation studies were carried out for pure drug curcumin and F7 formulation in phosphate buffer pH 6.4 using egg membrane by using Franz diffusion cell. The cumulative % drug released was found to be 46.6% in formulation and in pure drug it was about 75.8% after 6 hrs. Zero order and first order plots were constructed. The r^2 values were obtained as 0.740 and 0.885 for formulation and pure drug respectively in case of zero order plots and in case of first order plots it was found 0.799 and 0.928 respectively. The r^2 values of first order plots are more compared to zero order plots. Therefore based on these drug release studies it was concluded that the rate of drug release followed first order and was slow from the formulation compared to pure drug

CONCLUSION

Structured emulsion was prepared using oleocanthal enriched EVOO and white soft paraffin as oil phase. Sorbitol monostearate, emulsifying wax were used as emulsifying as emulsifying agents. Rheologfical properties were determined and the emulsion showed pseudoplastic and thixotropic behavior. Rate of drug release followed first order drug release. FTIR studies revealed the absence of drug excipient interactions.

REFERENCES

1. Emily W and Brian L. Active components and clinical applications of olive oil. Alternative medicine review. 12, 2007, 331-342.

2. Ana HM, catarina OS, Marisa N, Andre B, Lidia P and Patricia R. Design and evaluation of formulation with olive oil as natural functional active. Pharmaceutical development and technology. 2017, doi:10.1080/10837450.2017.1340951.
3. Lisa Parkinson and Russell Keast. Oleocanthal, a phenolic derived from virgin olive oil: A Review of the beneficial effects on inflammatory disease. International journal of molecular sciences 2014, 15, 12323-12334.
4. Simon Vassiliadis. Immune and homeostatic surveillance are positively modulate in cancer-like and cancer conditions by a number of natural products. Functional food in health and disease 2015, 5, 331-338.
5. Evangelia K, Eleni M, Prokopios M. Direct Measurement of Oleocanthol and Oleocein levels in olive oil by quantitative H-1 NMR. Establishment of a new index for the characterization of Extra Virgin Olive oils. J agricultural and food chemistry.2012 A-H.
6. Malay KD and Abdul BA. Formulation and ex vivo evaluation of rofecoxib gel for topical application. Acta Poloniae Pharmaceutica Drug research 63, 2007, 461-467.
7. William O and Vida OE. Evaluation of saponification value, iodine value and insoluble impurities in coconut oils from jomoro district in the Western region of Ghana. Asian journal of agricultural and food sciences. 2015, 3, 494-499.
8. Codex alimentarius, International food standards. Food and Agriculture organization o the United nations. www.codexalimentarius.org.
9. Evangelia K, Eleni M, Prokopios M. Direct measurement of oleocanthal and oleocin levels in olive oil by quantitative H-1NMR. Establishment of a new index for the characterization of extra virigin olive oils. Journal of agricultural and food chemistry 2012.
10. Alka G, Deepika A, Sanjay G and Anil KS. Spreading of semisolid formulations an update. Pharmaceutical technology, 2002. www.pharmatech.com
11. Rheology. Martin's Physical Pharmacy and Pharmaceutical sciences,6th Edition, Ed; Patric J Sinko and Yashveer Singh. Wolters Kluwer/Lipincott Williams & Wilkins;2006: 469-491.
12. John Coates. Interpretation of infrared spectra, a practical approach. Encyclopedia of analytical chemistry. R.A. Meyers (Ed.) Copyright © John Wiley & Sons Ltd.