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FORMULATION, OPTIMIZATION AND EVALUATION OF ACECLOFENAC TRANSDERMAL GEL- A NOVEL APPROACH FOR PENETRATION ENHANCEMENT BY HERBAL EXTRACT

Singh Rampal[1]*, Juyal Divya[1], Singh Vikram[1] and Rawat Geeta[2]

[1]Himalayan Institute of Pharmacy and Research, Rajawala, Dehradun.
[2]H.N.B.G.U, Department of Pharmaceutical Science, Srinagar Garhwal.

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***Correspondence for Author**
Singh Rampal
Himalayan Institute of Pharmacy and Research, Rajawala, Dehradun.

ABSTRACT

The present research has been undertaken with the ambition to formulate, optimize and evaluate the gel containing *acorus calamus oil*. The gel was prepared by using aceclofenac (API) and optimum ratio of carbopol 941 P and HPMC K15 M and other excipients are propylene glycol, triethanolamine, and methyl paraben. The aceclofenac permeability through stratum corneum occurs by using oil which obtained from the leaves of *acorus calmus*. The *acorus calamus oil* was used as a penetration enhancer. All prepared gel formulations were evaluated by pH, spredability, extrudability, viscosity, drug contents, *in- vitro* permeability studies and drug- polymer compatibility (FTIR studies). The absorption maxima of aceclofenac were found at 273 nm using ph 6.8 phosphate buffer solutions. The pH values of all formulation was in the range between 6.80 -7.23. Drug content of all formulation ranges from 82.72 - 90.22. The result value of spreadability was range from 11.66 - 14.66(gm. cm / sec). Whereas the extrudability value of gel formulations from the collapsible tube varies range from 250.00 to 444.44(gm.cm^2) and the viscosity of formulations ranges from 21023 cps to 22101 cps at 50 rpm. The % cumulative drug release range from 48.50 -66.78% in 6 hr.

KEYWORDS: A*corus calamus*, API, FTIR, *in- vitro* permeability studies.

INTRODUCTION

The Non-steroidal anti-inflammatory Drug (NSAIDs) possesses anti-inflammatory, analgesic and antipyretic activities. Aceclofenac is Non-steroidal Anti-inflammatory drug and chemically it is [(2,6Dichlorophenyl) amino] phenyl acetyloxyacetic acid. It widely used in

the treatment of pain (headache, back pain, arthritis and joint pain) act by inhibiting prostaglandin synthesis, which is cause of inflammation.[1, 2] Aceclofenac belongs to BCS class-II, it possess solubility problem for drug delivery. Aceclofenac with oral therapy causes dyspepsia, abdominal pain, nausea and diarrhea other rare side-effects include dizziness, constipation, vomiting, ulcerative stomatitis, rash, dermatitis, headache, fatigue.[3, 4]

Acorus calmus also known as sweet flag and buch that belonging from araceae family. [5] The plant has a characteristic essential oil called as the asarone oil. β-asarone (isoasarone) is usually the major constituent (27.4-45.5%) α-Asarone, β-asarone have a relaxing effect on smooth muscle tissue.[6, 7]

MATERIALS

1. **Plant material collection and authentification –** plant was collected from pauri garhwal, Uttarakhand at pond side during January month. Plant was identified by comparing with their authentic specimens with the help of taxonomists (Dr. Anup Chandra) Scientist-D, Forest Research Institute [FRI] Dehradun, Uttarakhand.
2. **Chemicals-** Aceclofenac [Tirupati Medicare Ltd, Paota Sahib (Himanchal Pradesh) India] carbopol 941 and HPMC K15M are propylene glycol, triethanolamine, and methyl paraben [Central Drug House (p) Ltd, New Delhi] Hifenac gel The Madras Pharmaceuticals, Chennai.

METHOD

A. EXTRACTION OF OIL -300 gm of dried crushed plant material of *acorus calamus* was taken in 1000 ml of round bottom flask and extracted with distilled water for 6- 8 hr at 70- 80°C using Clevenger apparatus and added a small porcelain chip to avoid bumping, the oil was collected which was light yellow in colour with sweet volatile odour.[8]

B. PHYTOCHEMICAL TESTS

ACORUS CALAMUS [9]

i. Test for Carbohydrate- Molish test

1ml of test solution with few drops of α-napthol. Add 0.2 ml of concentrated sulphuric acid slowly added from the side of tube, purple to violet colour ring appears at the junction.

ii. Protein- Biuret test

take 0.2 ml of test solution in test tube, add few drops of 4% sodium chloride solution and few drops of 1% copper sulphate, violet colour produce that indicate the presence of protein.

iii. Saponins- Forth test

take a pinch of coarsely dried powder of plant in test tube and add distilled water, the mixture was shaken, then the form produce which indicate presence of saponin.

iv. Terpenoids test- libermann-burchard test

few drops of acetic anhydride add into 0.5 ml of plant extract heat, boil and cool, add few drops of concentrated sulphuric acid with drop wise, a red colour was produced due to presence of terpenes.

v. Test of fixed oils and fats- spot test-

a small quantity of plant extract was pressed between two filter paper. Oil stain on the filter paper indicates the presence of oils and fat.

C. DISPERSION METHOD

Disperse the carbopol 941 and HPMC K15 M in distilled water and soaking for 8 hr. dissolve the drug in propylene glycol, and slowly mixed into the polymer solution with continuous stirring and addition of penetration enhancer, methyl paraben then pH adjustifier triethanolamine was added that modify the buffering capacity of gel. [10]

D. EVALUATION PARAMETER OF GEL

a. **Measurement of pH-**The pH of various gel formulations was determined by using digital pH meter. 1gm of gel was dissolved in 100 ml distilled water and stored for 2 hr. The readings were taken for average of 3 times.[11]

b. **Spreadibility** -0.5g gel was placed within a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5min. The increase in the diameter due to spreading of the gels was noted.[12]

c. **Extrudability-** a good gel extrudes from the tube with slight pressure applied. The extrudability of formulations from aluminium collapsible tubes was determined using universal tube filling machine. Aluminium collapsible tubes filled with 20g gels and tube

was compressed and extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5 cm. ribbon of gel in 10 seconds.[13]

d. Drug content- A 100 mg of developed gel dissolved in 100 ml of phosphate buffer with pH 6.8. The volumetric flask containing gel solution was shaken for 2 hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometrically at 273 nm using phosphate buffer (pH 6.8) as blank.[14]

e. Viscosity-The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer. The gels were rotated at 50 rotations per minute. 50 gm of preparation was kept in 50 ml beaker which was set till spindle groove was dipped and rpm was set and dial reading was measured after three minutes. From the reading obtained, viscosity was calculated by using factor. The readings were taken for average of 3 times.[15, 16]

f. *In-vitro* diffusion studies- The *in vitro* diffusion studies of prepared gel were carried out in Franz diffusion cell using through a cellophane membrane 10 ml of phosphate buffer solution (pH 6.8) was used as receptor compartment, then 1 mg of gel was spread uniformly on the membrane. The donor compartment was kept in contact with a receptor compartment and the temperature was maintained at 37±0.5°C. The solution on the receptor side were stirred by externally driven Teflon coated magnetic bars at predetermined time intervals, pipette out 3ml of solution from the receptor compartment and immediately replaced with the fresh 3ml phosphate buffer for 8 hr. Then the samples were analyzed by spectrophotometrically at 273 nm using phosphate buffer (pH 6.8) as blank for drug release.[17, 18]

g. Stability studies-The stability studies were carried out for all the gel formulation by, by subjecting the product to a temperature at 4° C, 25°C, 40°C for 15 days, The formulation was analyzed for the change in appearance, pH or drug content. [19, 20]

RESULTS

A. EXTRACTION OF OIL

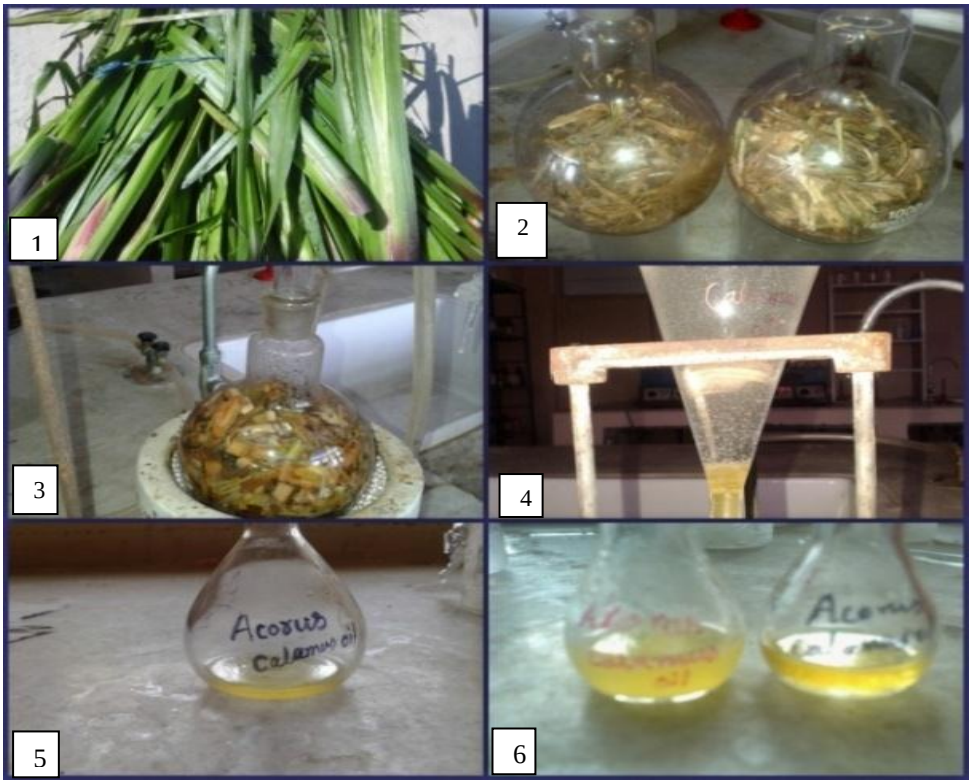


Figure 1. Extraction processes of *acorus calamus* (1. Plant collection, 2. Dried plant sample 3. Extraction of plant 4. Separation of oily portion, 5, 6. Collected oil.)

B. PHYTOCHEMICAL TESTS OF *ACORUS CALAMUS*

Table 1. Phytochemical tests of *Acorus calamus*

S. No.	Constituents	Tests performed	Results
1.	Carbohydrate	Molisch's test	+
2..	Proteins & amino acid	Biuret test	+
3.	Saponins	Forth test	+
4.	Fixed oil and fat test	Spot test	+
5.	Triterpenoids	Libermann burchard test	+

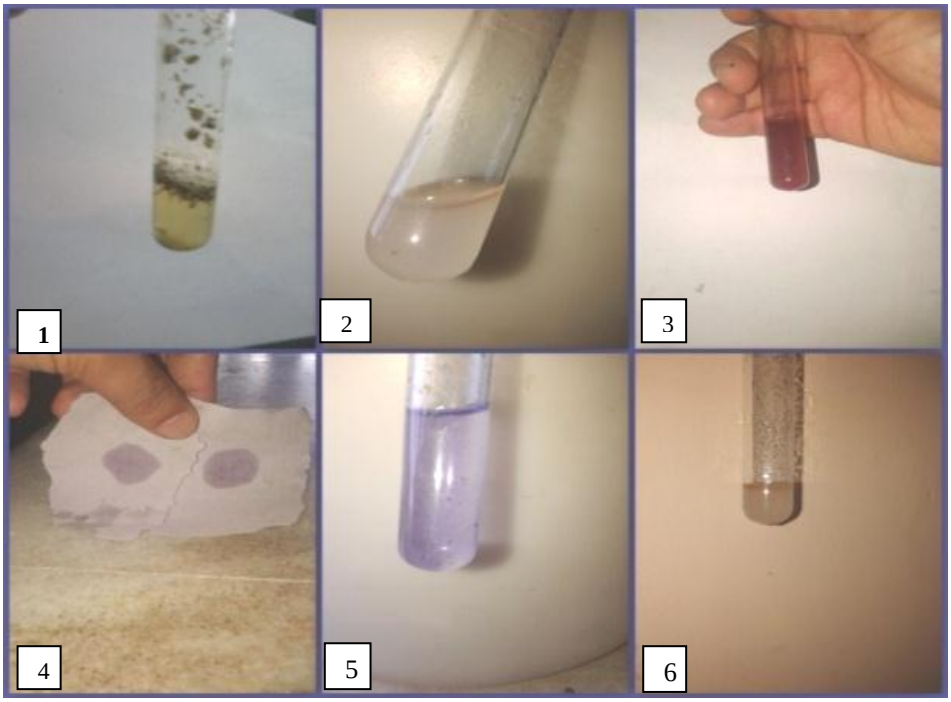


Figure 2. Phytochemical tests of *Acorus calamus* (1.Forth test 2.Molish test 4.Liberman burchard test 4.Spot test 5.Biuret test 6.Molish test)

C. DRUG IDENTIFICATION STUDIES-

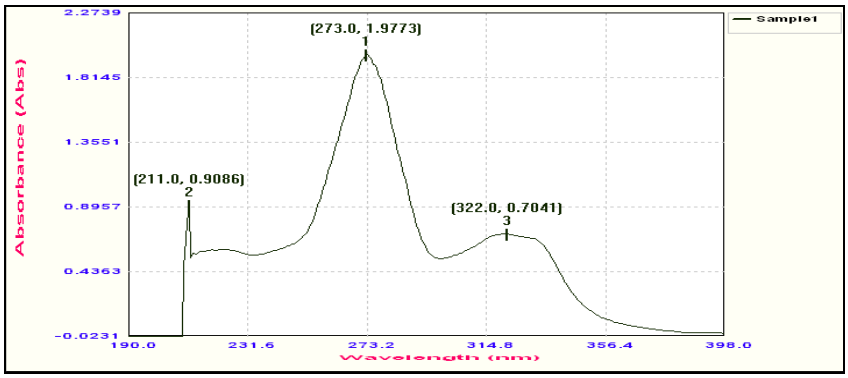


Figure 3. Absorption maxima (λmax) of aceclofenac in phosphate buffer pH 6.8

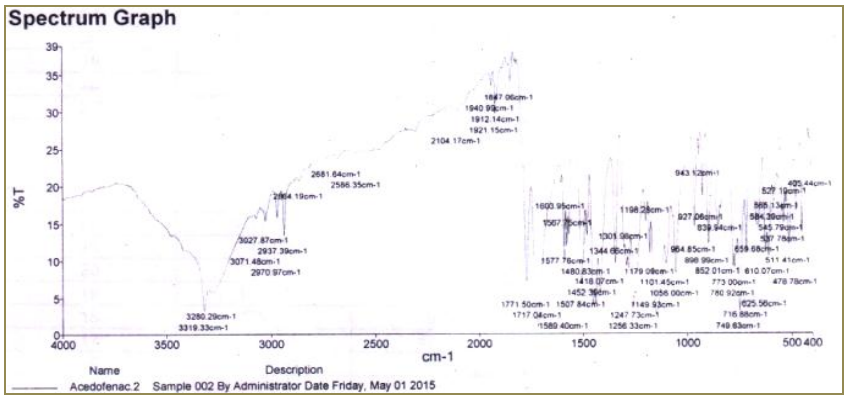


Figure 4. FTIR spectroscopy of aceclofenac sample

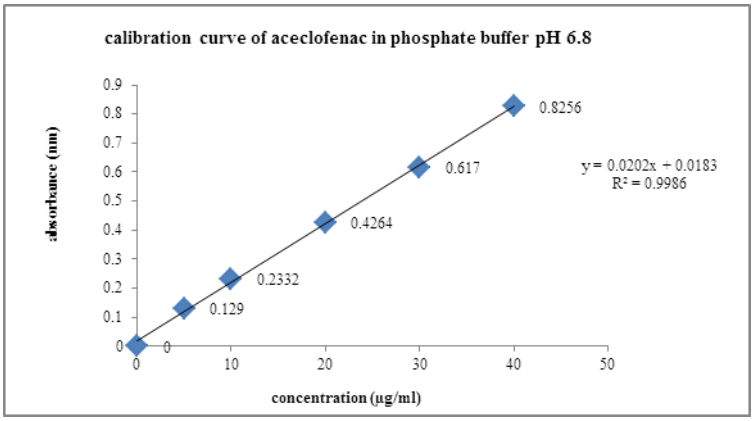


Figure 5. Calibration curve of aceclofenac in phosphate buffer solution pH 6.8

D. COMPATIBILITY STUDY OF GEL BY FTIR (NUJOL METHOD)-

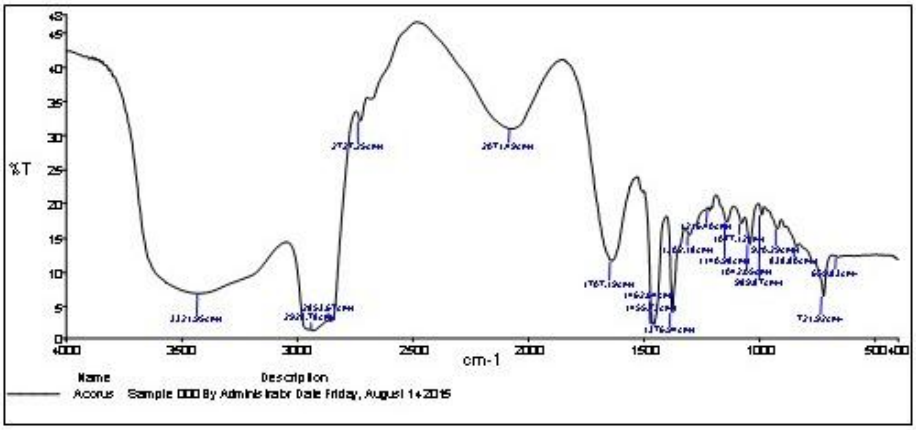


Figure 6. FTIR of aceclofenac gel contain *acorus calamus* oil

E. PREPARATION AND EVALUATION OF GEL

Table 2.Preparation of gel contain *acorus calamus*

S.NO.	INGREDIENTS	F1	F2	F3	F4	F5
1.	Aceclofenac(gm)	1	1	1	1	1
2.	Carbopol 941 P(gm)	1	1	1	1	1
3.	HPMC K15(gm)	1.5	1.5	1.5	1.5	1.5
4.	Methyl paraben (gm)	0.2	0.2	0.2	0.2	0.2
5.	Propylene glycol(ml)	10	10	10	10	10
6.	Triethanolamine(ml)	1	1	1	1	1
7.	*Acorus calamus* oil (ml)	1	1.5	2	2.5	3

Table 3. Evaluation data of gels containing *Acorus calamus*

Formulation code	pH	Spreadability (gm.cm/sec)	Extrudability(g m.cm^2)	Drug content (%)	Viscosity (cp)
AF1	6.92	12.33	333.33	89.3%	21023
AF2	6.80	11.66	285.71	86.67%	21786
AF3	6.98	13.66	250.00	82.72%	21621

AF4	7.06	13.00	444.44	88.55%	22004
AF5	7.23	14.66	307.69	90.22%	22101
Plain gel	7.02	10.33	400.00	81.39%	23260
Hifenac gel	6.69	11.66	250.00	90.57%	22150

Table 4. *Invitro* drug release data of developed gel aceclofenac gels in phosphate buffer solution pH 6.8

Time (hr)	*Acorus calamus* formulation % Cumulative release					Plain gel	Hifenac gel
	AF1	AF2	AF3	AF4	AF5		
0	0	0	0	0	0	0	0
1	1.82	2.60	2.91	1.85	2.01	1.39	2.55
2	6.11	7.15	8.02	5.92	7.54	3.74	7.21
3	12.84	13.35	16.64	13.53	17.10	6.95	16.35
4	22.30	26.74	29.12	25.32	30.47	11.13	29.52
5	33.54	37.23	43.87	41.88	46.94	15.82	47.91
6	48.50	53.21	60.40	62.52	66.78	21.00	69.69

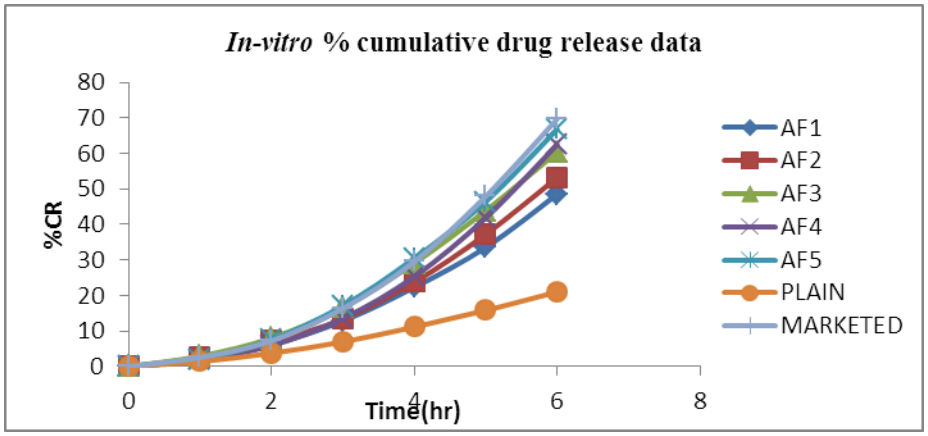


Figure 7. In-vitro %cumulative drug release data

F. *IN-VITRO* DRUG RELEASE KINETICS STUDIES

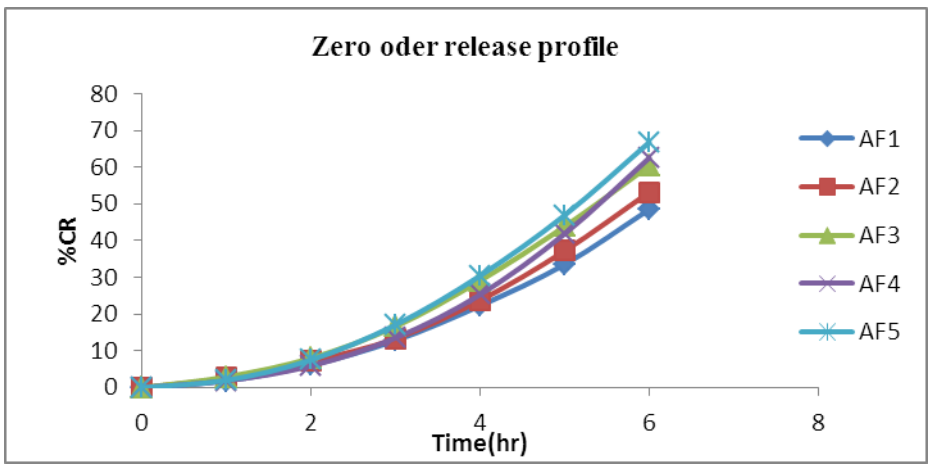


Figure 8. Zero order release profile of aceclofenac gel containing *acorus calamus*

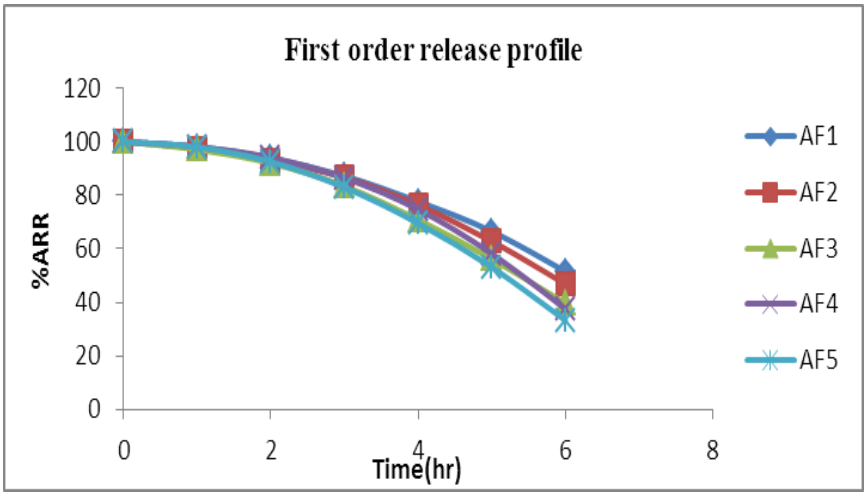


Figure 9. First order release profile of aceclofenac gel containing *acorus calamus*

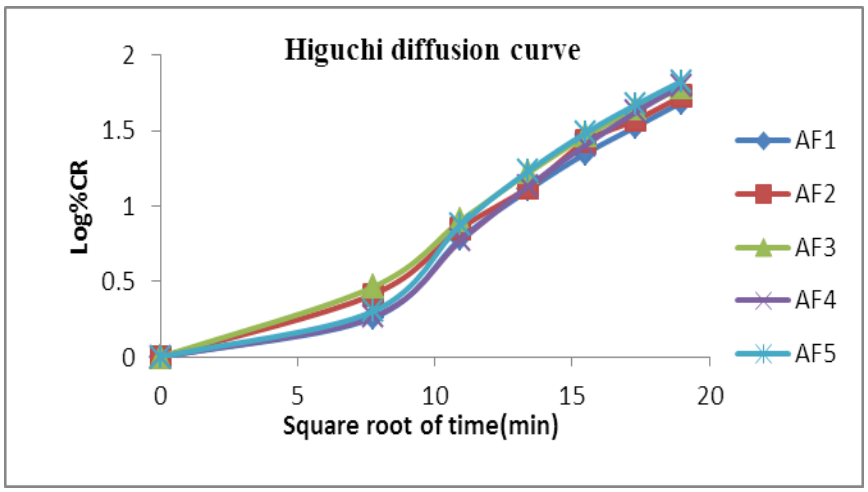


Figure 10. Higuchi diffusion curve of aceclofenac gel containing *acorus calamus*

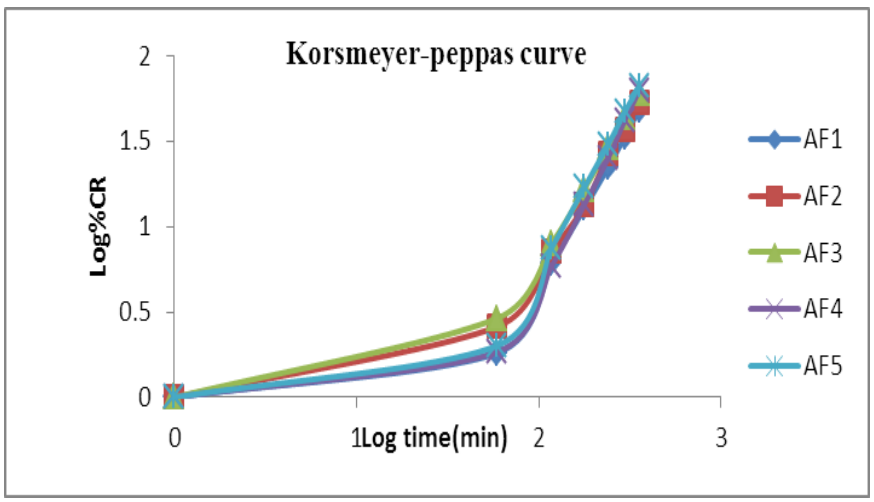


Figure 11. Korsmeyer-peppas curve of aceclofenac gel containing *acorus calamus*

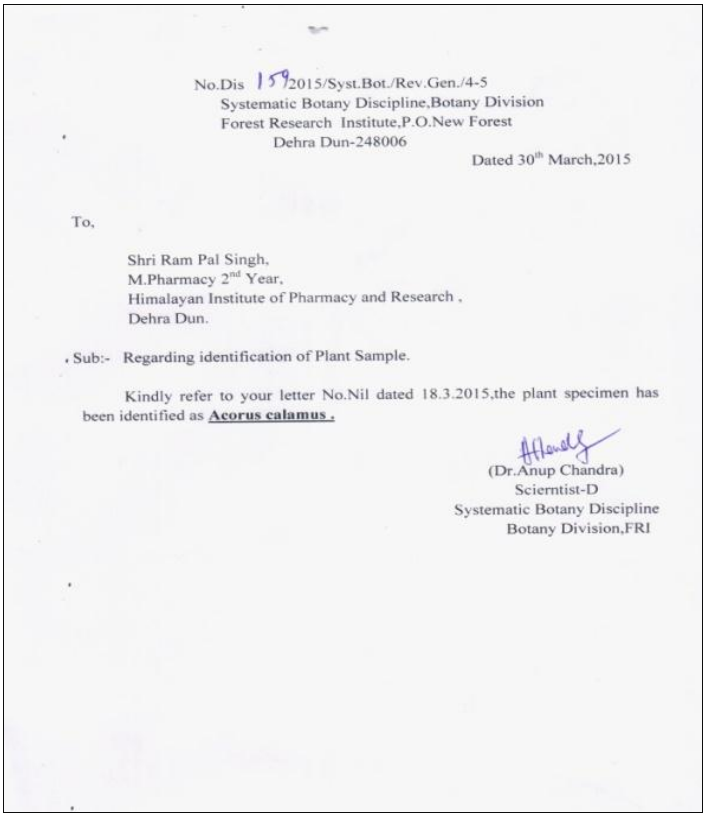
Table 5. Mathematical modelling and drug release kinetics of aceclofenac gel

Formulation code	*Acorus calamus*			
	Regression coefficient(R^2)			Slope (n) value
	Zero order	First order	Higuchi's	Korsmeyer-peppas
AF1	0.931	0.931	0.944	0.632
AF2	0.925	0.925	0.972	0.632
AF3	0.940	0.939	0.977	0.661
AF4	0.900	0.900	0.938	0.667
AF5	0.927	0.927	0.948	0.692
Marketed	0.912	0.912	0.963	0.680
Plain gel	0.965	0.965	0.924	0.832

G. STABILITY STUDIES

Table 6. Stability studies data of aceclofenac gels after 45 days

Formulation code	*Acorus calamus*	
	pH	% Drug content
AF1	6.91	85.72
AF2	6.73	80.20
AF3	6.70	86.00
AF4	7.01	80.62
AF5	7.12	87.47



No.Dis 159/2015/Syst.Bot./Rev.Gen./4-5
Systematic Botany Discipline,Botany Division
Forest Research Institute,P.O.New Forest
Dehra Dun-248006
Dated 30th March,2015

To,

Shri Ram Pal Singh,
M.Pharmacy 2nd Year,
Himalayan Institute of Pharmacy and Research ,
Dehra Dun.

Sub:- Regarding identification of Plant Sample.

Kindly refer to your letter No.Nil dated 18.3.2015,the plant specimen has been identified as **Acorus calamus .**

(Dr.Anup Chandra)
Scierntist-D
Systematic Botany Discipline
Botany Division,FRI

Figure 12. Plant authentification certificate of *acorus calamus*

DISCUSSIONS

The all formulated gels are white or translucent in appearance. The pH value of all formulated gels lies in between 6.80 -7.23. Drug content of all formulation ranges from 82.72 - 90.22%. The result values of spreadability indicate that the gel is easily spread over skin surface by small amount of shear. The result of spreadability range from 11.66 - 14.66 (gm. cm / sec). whereas the extrudability of gel formulations from the collapsible tube varies from 250.00 to 444.44 gm.cm^2 and viscosity of formulations ranges from 21023 cps to 22101 cps at 50 rpm. The % cumulative drug release range from 48.50 -66.78% in 6 hr.

CONCLUSSION

In- vitro drug release study results show the formulations containing *acorus calamus*, the drug releasement are faster as compared to plain formulation of gel which does not contain *acorus calamus*. It may be concluded from the results that as the concentration of *acorus calamus* 1 to 5 ml (48.50-66.78 %CR) increases in the formulations the rate of drug release also increases. Percent drug release data also show the formulation of gel that contains *acorus calamus* oil (F5-5ml) a best formulation that is similar to marketed formulation (Hifenac gel). In which the drug release 45.78% more after addition of penetration enhancer compare to plain gel in which only 21.00% drug was release. The mathematical kinetic studies is showed the value of correlation coefficient (r^2) 0.948 *(acorus calamus)* in case of higuchi diffusion model and this value was supported by n value i.e. 0.692*(acorus calamus)* korsmeyer peppas (in the literature 0.5 n value was perfect fickian diffusion but after in various literature 0.5 to 0.6 believe to be fickian types of diffusion). So we can state that the selected formula of gel release entrapped amount of drug by diffusion way which was more or less ficknian types. It is clear that *acorus calamus* can significantly enhance the penetration of aceclofenac from gel formulation across the skin. The usage of herbal oil was found to be efficient in releasing of drug.

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