

PREPARATION AND EVALUATION OF POLYHERBAL NANOSYSTEM FOR CANCER TREATMENTS

Ravindra A Sarkate*, Research Scholar, Dept. of Pharmacy, SunRise University, Alwar
Dr.Nandu Kayande**, Professor, Dept. of Pharmacy, SunRise University, Alwar, Rajasthan, India

ABSTRACT:

Background Herbal drugs which are shows anticancer activity are selected for present study such as, *allium sativum* (Garlic), *Azardirectaindica* (Neem), *Currcuma longa* (termeric), and *Nigella sativa* (black seed oil). **Aim:** The aim of this study was to prepare and evaluate Herbal liquid for hard gelatine capsule. **Methods:** The liquid for hard gelatine capsules were prepared by using PEG 400, propylene glycol and water mixture under temp of 90⁰ C followed by adding PVA, Cremophore EL, Labrasol and in last herbal extract finally added to the mixture under stirring. Final formulation was filled in hard gelatin capsule and hand band seal was performed. **Physico-Chemical Evaluation:** Total nine liquid filled hard gelatine capsule formulations were prepared (F-1 to F-9) and evaluated for weight variation test, lock length, drug content, nephelometric turbidity units (NTU) and in vitro release study. **Results:** The drug content ranged between 92.760.12 and 99.25 0.19. The results showed that the medication content is consistent across all formulations. The disintegration time varies between 130 and 232 seconds. According to an in-vitro dissolution research, the percent cumulative drug release ranges from 84.101.766 to 99.570.653. The optimised formulation F7 was shown to be stable for up to 30 days at 40 2oC and 75 5% RH. **Conclusion:** Hence liquid filled hard gelatine capsule of herbal extract can be a potential alternative to available traditional oral drug delivery systems to improve its solubility.

Keywords: Liquid filled hard gelatin capsule, traditional oral drug delivery systems, Herbal extract.

INTRODUCTION:

Capsules were utilised because they were easy to produce as unit dose forms for medications in powdered or granular form and provided an easy-to-swallow container that successfully concealed the harsh taste of drugs. 1-4 With the advent of pellet technology, which allowed for modified drug release, capsules became a useful vehicle into which multiparticulates could be filled without the risk of modifying the release characteristics associated with other processing methods, such as compressing multiparticulates into tablets. Since the early 1980s, technology has allowed for precise dosage and sealing of liquids within hard gelatin capsules.⁵⁻⁷

Gelatin, sugar, and water are used to make the empty capsule shells. As a result, they can be transparent, colourless, and tasteless, or they can be tinted with various dyes and made opaque by adding substances like titanium dioxide. 4 Most commercially available medicinal capsules have colourant and opaquant combinations to distinguish them, with many having different coloured caps and bodies. Gelatin is made by partially hydrolyzing collagen from animal skin, white connective tissue, and bones. 4 It is commercially available in fine powder, coarse powder, shreds, flakes, and sheets. Gelatin dissolves quickly in hot water and warm stomach fluid, exposing the contents of a gelatin capsule.⁸⁻¹⁰

MATERIALS AND METHOD:

Preparation of Liquid filled hard gelatin capsule

1. Preparation of liquid formulation F7 to be filled in hard gelatin capsule

Method:

To a container PEG 400 (10%), propylene glycol (10%) and water (3%) were added under stirring and heating. Product temperature was maintained at 90⁰. To the above step slowly PVA (4%) was added under stirring and product temperature was maintained at 90⁰C. To the above solution Cremophore EL (44%), Labrasol (17%) were added under stirring and heating. To the above step, Herbal extract (12%) was finally added to the mixture under stirring and heating at 90⁰C.

Final formulation was filled in hard gelatin capsule and hand band seal was performed.

Table 1:Composition of prepared liquid herbal extract

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Herbal extract	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Propylene Glycol	21.6	21.6	40.0	21.6	21.6	21.6	21.6	21.6	21.6
PEG 400	21.6	21.6	40.0	21.6	21.6	21.6	21.6	21.6	21.6
Water	5.8	5.8	10.0	5.8	5.8	0.0	0.0	0.0	0.0
Labrasol	70.0	70.0	70.0	70.0	35.0	35.0	35.0	35.0	35.0
Gelucire 44/14	120.0	0.0	0.0	144.0	0.0	0.0	0.0	0.0	0.0
Cremophore EL	0.0	120.0	0.0	0.0	90.0	90.0	0.0	90.0	90.0
PVP K-30	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0
2N Hcl	0.0	0.0	0.0	0.0	0.0	12.7	1.2	0.0	3.4
Oleic acid	0.0	0.0	0.0	0.0	0.0	0.0	30.0	29.0	29.0

Evaluation parameters of liquid filled capsules

- 1. Weight variation test:** All the filled capsules were subjected for weight uniformity test. Here, each individual capsule was calculated by adding up its weight for empty capsule shell weight in mg, liquid weight to be filled in hard gelatin capsule and band seal weight. In this test 20 units of capsules were weighed individually and average weight was calculated from which percentage deviation was determined.¹¹
- 2. Lock length:** Lock length is the size height of capsule shell from one end to locking area of cap and body of capsule shell. Lock length can be used to determine the capsule size, empty capsule weight and its filling capacity by using formula $M = V \times D$ (M = Mass, V = Volume and D = Density of contents). The capsule lock lengths for individual formulation were measured using vernier caliper.¹²
- 3. In vitro Release Study:** The *in vitro* release of drug from liquid filled hard gelatin capsules were carried out for 45 minutes using dissolution apparatus USP type II. Liquid filled in hard gelatin capsules, which consists of drug equivalent to 25 mg. We have studied the release for every 15 minutes in 6.8 phosphate buffer was used and was replaced with same pH 6.8 phosphate buffer for every 15 minutes till 45 minutes of release. The dissolution media was maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and a speed of 50 rpm. At prefixed time interval (every 15 minutes), 5 ml of solution was withdrawn and replaced with 5 ml of fresh buffer solution. After suitable dilutions, the samples were analyzed at 282 nm with phosphate buffer using UV-1800 series spectrophotometer.¹⁴
- 4. Statistical Analysis**
Statistics is a logic, which makes use of mathematics in the science of collecting, analyzing and interpreting data for the purpose of making decisions. Now, after many evaluations carried out on all the formulations of liquid filled in hard gelatin capsules the data obtained were subjected to statistical analysis of formula F7. A computer aided calculations were done by using a preprogrammed software.¹⁵
- 5. Nephelometric turbidity units (NTU):** NTU are based on white light (400–680nm) and 90° incident angle. Many liquids are essential in our daily lives, such as water, chemicals, acids, bases or pharmaceutical products such as our samples. The quality of these liquids is determined by their chemical and physical properties. To assess these properties various principles of measurement are used as in case of our research study. One of these principles is measurement of turbidity in liquid. Turbidity is the cloudiness caused by suspended particles of liquid. These particles scatter the incident light and the liquid loses its transparency. The instrument used was digital Nephelometer, it has holder for glass tube with lid to cover, where we start by keeping the standard samples for calibration. Other segment is for power where we run the experiment by switching the mode towards on segment. The calibration is performed by using our standard sample/s that is distilled water, so that the instrument is

calibrated. Then we use our samples for analysis to measure the turbidity and the turbidity of our sample/s is measured as shown on nephelometer in NTU.¹⁶

6. Correlations

Coefficient of correlation (r) was calculated with the help of instat. The 't' values for 't' test of significance were also calculated. Considering the linear correlation $y=bx+a$, the b value (slope) and a value (y-intercept) were obtained.¹⁷

Model fitting for release of Herbal liquid from liquid filled hard gelatin capsule:

The different drug release profiles were calculated and analyzed for best fit models by incorporating the data into:

1. Zero Order
2. First Order
3. Higuchi
4. KorsmeyerPeppas

RESULT AND DISCUSSION

S.N.	Conc ppm	Absorbance in Methanol	Absorbance in pH 6.8
1	21	0.472	0.264
2	14	0.581	0.108
3	7	0.200	0.254
4	3.5	0.541	0.034
5	1.4	0.718	0.016
6	0.7	0.02	0.004
7	0.14	0.026	0.007

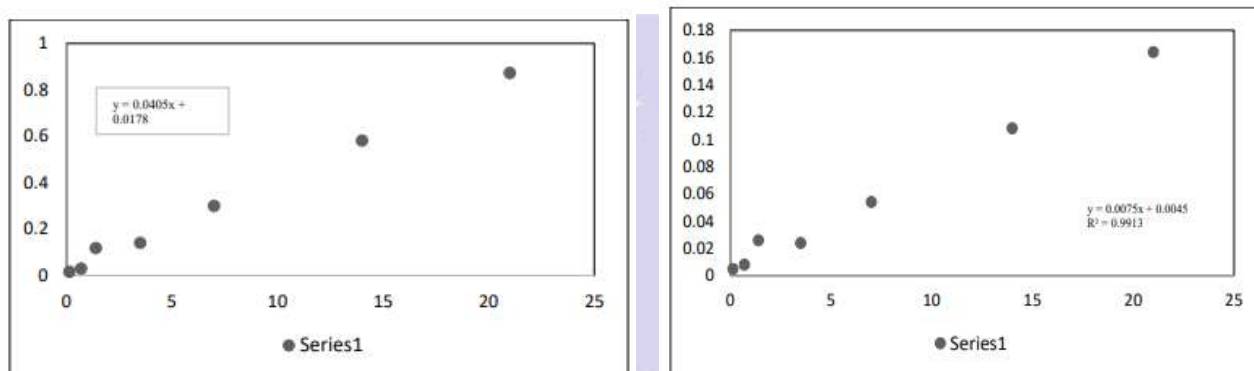


Figure 1: Standard Calibration Curve of herbal liquid in pH 6.8 Phosphate buffer ($\lambda_{max}=282$ nm)

Table 1: Physicochemical Parameters of liquid filled hard gelatin capsules

Formulation Code	Lock length Avg (mm)	Disintegration time (sec)
F1	14.19+0.07 mm	210
F2	16.19+0.07 mm	230
F3	15.35+0.04 mm	232
F4	17.19+0.07 mm	180
F5	16.35+0.04 mm	150
F6	16.19+0.07 mm	172
F7	17.35+0.04 mm	160
F8	13.19+0.07 mm	170
F9	12.19+0.07 mm	158

Table 2: Dissolution Profile of Herbal liquid from formulation F1-F9

Formulations	Time point		
	15 min	30 min	45 min
F1	78	88	89
F2	65	73	81
F3	81	90	93
F4	75	88	98
F5	98	99	99
F6	72	78	83
F7	79	84	86
F8	89	91	91
F9	88	92	97

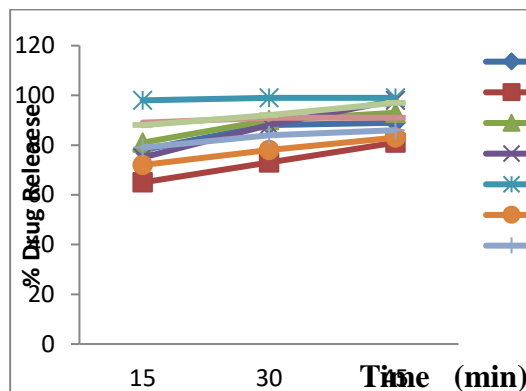


Figure 2: % drug release of liquid filled hard gelatin capsules (F1-F15)

Table 3: In Vitro Drug Release from formulation F7

Time (min)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released	% Drug released	Cube Root of % drug Remaining(Wt)
0	0	100	0.000	2.000	0.000	0.000	100	4.642
15	94.00	6	3.873	0.778	1.176	1.973	94	1.817
30	99.00	1	5.477	0.000	1.477	1.996	5	1.000
45	99.00	1	6.708	0.000	1.653	1.996	0	1.000

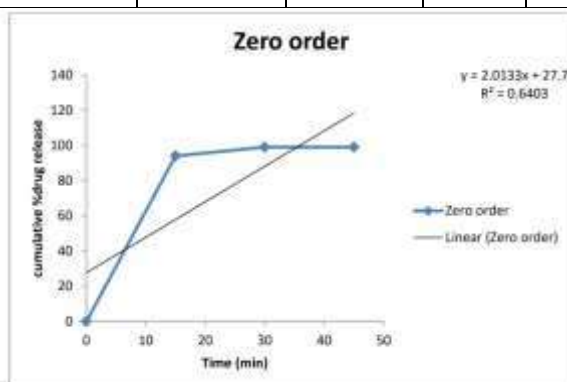


Figure 3: Zero order plots of liquid filled hard gelatin capsule (F7)

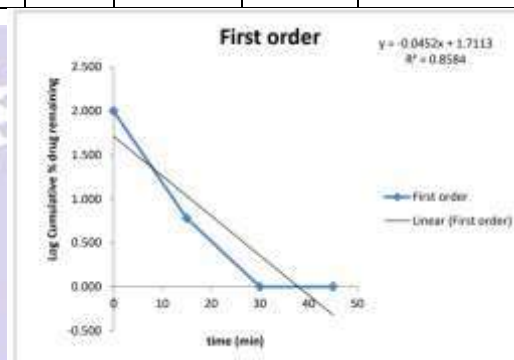


Figure 4: First order plots of liquid filled hard gelatin capsule (F7)

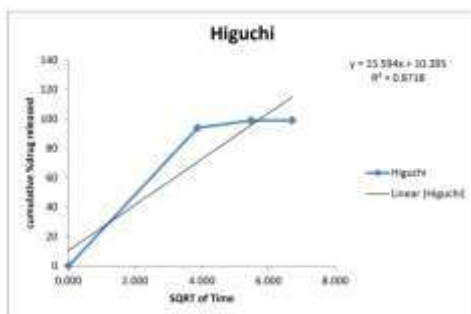


Figure 5: Higuchi's dissolution plots for liquid filled hard gelatin (F7)

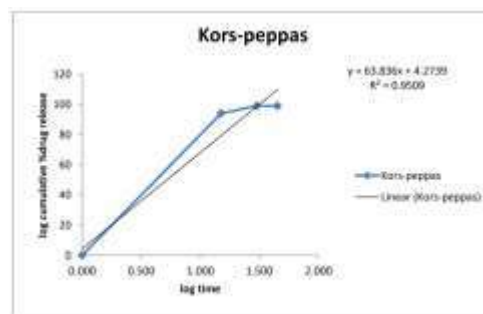


Figure 6: Korsmeyer-Peppas dissolution plots for liquid filled hard gelatin (F7)

Table 4: Nephelometric turbidity units (NTU)

S.N.	1	2	3	4	5	6	7	8	9
formula	F1	F2	F3	F4	F5	F6	F7	F8	F9
NTU (min to max)	635 to 757	692 to 785	221 to 310	710 to 884	593 to 661	584 to 637	546 to 607	511 to 623	587 to 603

#NTU are based on white light (400–680nm) and 90° incident angle.

On an electronic analytical balance, the weight fluctuation of each liquid-filled capsule was calculated as mean SD. Using liquid filled capsules containing Herbal liquid, the drug concentration of optimum batches was estimated. Three trials from each formulation are spectro-photometrically analysed. All formulations' mean value and standard deviation are computed. The drug content ranged between 92.760.12 and 99.25 0.19. The results showed that the medication content is consistent across all formulations.

The drug release investigations submitted to Korsmeyer'speppas' mathematical model were utilised to investigate the mechanism of drug release of the optimised batch (F7) of the liquid filled hard gelatin capsule. The correlation coefficient (r^2) was used as an indicator of best fit for each of the models. This diagram depicts the drug release procedure of a Herbal liquid liquid filled hard gelatin capsule. At 40 2oC and 75 5% RH, the optimised formulation F7 was shown to be stable for up to 30 days. There was no significant change in medication content or cosmetic appearance (colour changes). All formulations kept at higher temperatures showed very little change in disintegration time, drug content, and drug release. The drug release investigations that were subjected to the mathematical model of Korsmeyer'speppas were used to examine the mechanism of drug release of the optimised batch (F7) of the liquid filled hard gelatin capsule. For each of the models, the correlation coefficient (r^2) was utilised as an indicator of the best fit. The medication release process of a Herbal liquid liquid packed hard gelatin capsule is depicted here. The optimised formulation F7 was shown to be stable for up to 30 days at 40 2oC and 75 5% RH. There was no substantial change in drug content or aesthetic appearance (colour changes). Disintegration time, drug content, and drug release were all very little altered in all formulations held at increased temperatures.

ACKNOWLEDGEMENT: NIL

CONFLICT OF INTEREST: The authors declare no conflict of interest exists.

CONCLUSION:

Various oils were used to develop a liquid-filled hard gelatin capsule of herbal liquid. The capsule had the highest drug content and drug release (F7). Based on the results of our experiments, liquid filled hard gelatin capsules of Herbal liquid may be a good alternative to conventional marketed formulations, with potential improvements in drug absorption and subsequent bioavailability.

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