*Section A-Research paper*

# STUDY OF EFFECT OF SURFACE AREA AND DISSOLUTION METHOD ON IN-VITRO RELEASE OF DISULFIRAM IMPLANT

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## ABSTRACT

*Disulfiram is widely prescribed to discourage alcoholics from drinking alcohol. The effectiveness of oral disulfiram as a treatment for alcoholism is severely limited due to its poor bioavailability and poor patient compliance. To minimize the failure of the orally administered drug, efforts have been made to prepare alternative dosage form of subcutaneously implantable disulfiram pellets or tablets. In the present study an attempt has been made to design and evaluate a disulfiram implant using plain drug. It is known that variation in surface area and compression force used in pellet manufacture affects their densities or hardness. The disulfiram implants have been formulated by direct compression and the effect of surface area andtwo different method (S2-550lb/Cm$^2$, S6-1100lb/Cm$^2$, S10-1650lb/Cm$^2$&S14-2200lb/cm$^2$ with 9.98mm die & punch set) and (S3-550lb/Cm$^2$, S7-1100lb/Cm$^2$, S11-1650lb/Cm$^2$& S15-2200lb/cm$^2$ with 7.98mm die & punch set) were studied on in-vitro release of implantable disulfiram pellets. The release kinetic mechanism from all the formulation was found to be zero order. Two-way ANOVA & Tukey's multiple comparison test shows the release rate constants of formulation (S2, S6, S10& S14)&(S3, S7, S11&S15) obtained by two different methods i.e., (VM & RFM) are significantly different thus the effect of two different method & effect of surface area for all formulations is significant with p value <0.0001 ******

*Keywords: Disulfiram, Implant, Pellets, Topical drug delivery system,*

## 1.INTRODUCTION

Drug delivery systems that can sustain pharmacologically effective therapeutic drug levels for long periods of time while also permitting "dosing-on-demand" would be immensely useful in modern medicine. Physicians can choose from a variety of precision delivery options, such as local or systemic circulation, while still ensuring appropriate dose over the duration of treatment with implantable drug delivery systems. These systems have several advantages, including focused local medication delivery at a steady and predetermined pace, which reduces the amount of drug required and potential side effects while boosting therapeutic efficacy. These systems are especially useful for conditions including cardiovascular disease, tuberculosis, diabetes, cancer, and chronic pain management, to mention a few, that require long-term medication or face issues with patient compliance. The problem behind the usefulness of disulfiram is widely prescribed to discourage alcoholics from drinking alcohol, since alcohol and disulfiram interact to produce a subjectively unpleasant experience characterized by facial flushing, nausea, tachycardia and hypotension etc"DER / DAR" Reaction [1,2]. The effectiveness of oral disulfiram as a treatment for alcoholism is severely limited due to its poor oral bioavailability and by the willingness of patients to take the drug every day, many stop taking their tablets so that they might resume drinking alcohol as soon as the effect have worn off. So, the frequent failures with the orally administered drug have stimulated interest in parenteral therapy with subcutaneously implanted disulfiram pellets.

The objectives of the proposed research work are developing an implant, which will be clinically effective. The most preliminary approach in designing implantable pellets by directly compress plain disulfiram drug. The approach in designing of implant is directly compress the plain disulfiram at two different surface area i.e., 9.98mm & 7.98mm, the aim was to study the effect of surface area on the in vitro release of subcutaneously implantable disulfiram pellets by two different methods. The second approach is to study the effect of two different method (Vial method &Rotary Flask Shaker method) on in vitro release. Finally, an

attempt has to be made to predict the kinetics and mechanism of in vitro release from subcutaneously implantable pellets.

**4. MATERIALS AND METHOD**

The material for proposed work is Disulfiram USP, 0.2 M Sodium hydroxide, Potassium dihydrogen phosphate, Magnesium stearate, Copper (II) Chloride (dihydrate), Methanol AR, and Distilled water. The various apparatus to be used for study are as I.R. press (Lab India), U.V. spectrophotometer (Shimadzu UV-250 1PC double beam spectrometer), Rotary Flask Shaker. The proposed work optimized with software as NGSS, USA, Sigma-stat statistical software version 2.03 and Prism statistical software, and Microsoft Excel were used for the calculation, graphs and data treatment of the results obtained.

**Identification and Characterization of Drug sample disulfiram:** The drug sample was used without further purification and characterization of drug was done using physicochemical methods.

**Organoleptic properties and Description:** The sample of Disulfiram was studied for organoleptic characters and it was found to be a white or almost white, odorless and tasteless crystalline powder.

**Melting point:** The melting point was determined by Open Capillary Method and the uncorrected melting point was found to be 70 - $74^0$ C.

**Solubility:** The solubility of the Disulfiram was determined by adding excess amount of drug in the solvent and equilibrium solubility was determined by taking supernatant and analyzing it on Shimadzu UV 2501 PC, double beam, double monochromator spectrophotometer. The solubility studies have suggested the following values as in the **Table 1**. The drug was found to be slightly soluble in water and freely soluble in acetone and Tween-80.

**Analytical study by UV Spectroscopy:** A stock solution of Disulfiram in methanol of 20 µg/ml was prepared. To 5.0 ml of this solution 20.0 ml of 0.1% w/v solution of cupric chloride in methanol was added. The solution was thoroughly mixed and allowed to stand for 1.0 hour. The spectrum of this solution was recorded using Shimadzu UV 2501 PC, double beam, spectrophotometer at 1.0 nm slit width using methanol and water as solvent in the range of 300 – 600nm [3]. The wavelength of maximum absorption (λmax) was found to be 395.5 nm.

**Construction of Beer - Lambert's plot:** A standard curve was prepared by dissolving 10 mg of Disulfiram in 20 ml of methanol. It was further diluted with 0.1% w/v solution of cupric chloride in methanol to get the solution in range of 5 to 40 µg/ml. The absorbance of these solutions was determined spectrophotometrically at 395.5 nm [4,5].

**Preparation of Implants:** The active ingredient was made into desired pellets by direct compression at the respective compression force **Table 3**. All the Formulation were compressed using I.R. press quipped with 9.98 mm& 7.98 flat faced punch and die set. The compression force applied for 30 seconds (S2-550lb/$Cm^2$, S6-1100lb/$Cm^2$, S10-1650lb/$Cm^2$& S14-2200lb/$cm^2$ with 9.98mm die & punch set) and (S3-550lb/$Cm^2$, S7-1100lb/$Cm^2$, S11-1650lb/$Cm^2$& S15-2200lb/$cm^2$ with 7.98mm die & punch set) respectively. Before compression, the surfaces of the die and punch were lubricated with magnesium stearate.

**Evaluation of Implants:** The compressed implant matrix was evaluated for thickness, weight variation test, hardness and drug content[6].

**Thickness and Diameter variation Test:** The thickness of implants (n=6) was determined using a Micrometer Screw Gauge (Japan).

**Hardness Test:** For each formulation, the hardness of implants (n=6) was measured using the Monsanto hardness tester (Cadmach, Ahmedabad, India)

**Weight Variation Test:** To study weight variation, (n=20) pellets of each formulation were used.

**Drug Content:** Five implants were weighed and powdered. The drug content was measured as per the following compendial procedure.

**Standard Solution**: 40 µg/ml of disulfiram in 0.1% w/v solution of cupric chloride in methanol.

**Sample Solution**: An accurately weighed amount of powder equivalent to 0.4 gm of disulfiram was dissolved in 75.0 ml of methanol; this solution was adjusted to 100.0 ml with methanol. The 5.0 ml of the

resulting solution was again diluted to 100 ml with methanol. The solution was thoroughly mixed and filtered. To the 5.0 ml of the resulting solution sufficient 0.1% w/v solution of cupric chloride in methanol was added to produce 25 ml of the solution. The extinction of standard and sample solution was measured at 395.5 nm using blank solution prepared by diluting 5.0 ml ofmethanol to 25.0 ml with the cupric chloride solution. The results of evaluation of implants for thickness, weight variation, hardness and friability and drug content were shown in **Table 4**.

**Sterilization of Implant:** The formulation S1 was sent to well-known Bhaba Atomic Research Centre, Mumbai for gamma ray sterilization.

The radiation source used -Co-60

Duration of exposure – 5 to 7 minutes

Dose of Radiation – 2.5 Mrad which is equivalent to 25kGy (Kilogray)

**Sterility Test** [7]**:** Sterility test was carried out by using direct inoculation method. 20 units were directly transferred to sufficient volume of fluid thioglycollate medium. This fluid thioglycollate medium was incubated at 30 to 35 $^0$C for 14 days. Media were observed visually for any turbidity and microbial growth after 14 days.

**in vitro release study:** The experimental design for in vitro release studies was as given in table the in vitro release study was done by using two different methods first is Vial Method [8,9,10,11]& other is Rotary flask Shaker Method [12,13,14].

**Data Treatment:** Different Kinetic equations (zero-order, first-order and square root law of kinetic equation) were applied to interpret the release rate from all the formulations and as reported in **Table 8 & 9**. The best fit with higher correlation ($r^2$> 0.98) was found with the zero-order equation for all the formulation as shown in the **Table 8 & 9**. There are some factors, which diminish the applicability of zero order equation.

**Result and Discussion:** The characterization of Disulfiram was done by physicochemical parameters as well as by spectroscopic methods. The drug was found to be pure and was used in the study without any purification. Analysis of drug was done by compendial method for the entire work.

**Evaluation of implants**

**Drug Content:** All the implants had uniform distribution of drug in all the formulations. The drug content is as shown in the **Table 4**.

**Microbiological testing:** No visual growth of microorganisms was seen after 14 days incubation period on fluid thioglycollate medium suggesting the sterility of implant.

**Dissolution of Disulfiram Implant:** The dissolution data of all the formulation by Vial and Rotary Flask shaker Method are as shown in the **Table no. 6&7**. These data were treated with various dissolution models [15] to interpret and discuss the results obtained from the in-vitro release of different formulations of disulfiram Implants.

**Release Kinetics:** To gain better insight into the mechanism underlying the release of disulfiram from subcutaneous tissue implants and their role in systemic delivery of disulfiram, the release kinetics of disulfiram was investigated.The results were fitted to the zero order and first order model. The values of kinetic rate constant (K) and regression coefficient as calculated from zero order are shown in **(Table 8&9)**. From the regression coefficient it is clear that release of all the formulation by both the methods shows zero order kinectics. Hence for all the statistical interpretation, zero order release constants were selected. All the formulations contain pure drug which is very slightly soluble, obviously the best fit was obtained was for zero order. Higuchi square root and Korsemeyer peppas equations were not applied as no polymer was used in the formulations.

**Effect of surface area and method (S2 & S3):** The zero-order release rate constant data of formulation**S2&S3** obtained from the study of in vitro release by Vial method **(Table 10)** was subjected to two-way ANOVA (**Table 11)** followed by Tukey's multiple comparison test**(Table 12)**to study the effect of surface area and method. Two-way ANOVA & Tukey's multiple comparison test shows the release rate

constants of formulation S2 & S3 obtained by two different methods i.e., (VM & RFM) are significantly different thus the effect of two different method & effect of surface area for two different formulations (S2-9.98 mm & S3-7.98mm) is significant with p value <0.0001 **** **(Inference no. 1)**

**Effect of surface area and method (S6 & S7):** The zero-order release rate constant data of formulation**S6 & S7** obtained from the study of in vitro release by Vial method **(Table 13)** was subjected to two-way ANOVA (**Table 14)** followed by Tukey's multiple comparison test**(Table 15)**to study the effect of surface area and method. Two-way ANOVA & Tukey's multiple comparison test shows the release rate constants of formulation S6 & S7 obtained by two different methods i.e., (VM & RFM) are significantly different thus the effect of two different method & effect of surface area for two different formulations (S6-9.98 mm & S7-7.98mm) is significant with p value <0.0001 **** **(Inference no. 2)**

**Effect of surface area and method (S10 & S11):** The zero-order release rate constant data of formulation**S10& S11** obtained from the study of in vitro release by Vial method **(Table 16)** was subjected to two-way ANOVA (**Table 17)** followed by Tukey's multiple comparison test**(Table 18)**to study the effect of surface area and method. Two-way ANOVA & Tukey's multiple comparison test shows the release rate constants of formulation S10 & S11 obtained by two different methods i.e., (VM & RFM) are significantly different thus the effect of two different method & effect of surface area for two different formulations (S10-9.98 mm & S11-7.98mm) is significant with p value <0.0001 **** **(Inference no. 3)**

**Effect of surface area and method (S14 & S15):** The zero-order release rate constant data of formulation**S14& S15** obtained from the study of in vitro release by Vial method **(Table 19)** was subjected to two-way ANOVA (**Table 20)** followed by Tukey's multiple comparison test**(Table 21)**to study the effect of surface area and method. Two-way ANOVA & Tukey's multiple comparison test shows the release rate constants of formulation S14 & S15 obtained by two different methods i.e., (VM & RFM) are significantly different, the effect of two different method & effect of surface area for two different formulations (S14-9.98 mm & S15-7.98mm) is significant with p value <0.0001 **** **(Inference no. 4)**

**Effect of surface area& method:** To check the effect of surface area on in vitro release the in vitro release data of formulation **(S2, S6, S10 & S15 with 9.98mm diameter) & (S3, S7, S11, S15 with 7.98mm diameter)** was subjected to Two-way ANOVA followed by Tukey's multiple comparison test**(Table no. 10 to 21)**. The Two-way ANOVA followed by Tukey's multiple comparisontest shows that there is significant differences between **(S2, S6, S10 & S15 with 9.98mm diameter) & (S3, S7, S11, S15 with 7.98mm diameter)** formulation, i.e. with increase in surface area there is increase zero order release rate constants. From the statistical (**Inference no. 1,2,3& 4)** it was concluded that surface area had marked effect on in vitro release pattern of disulfiram implant. It was also observed that direct relationship exists between surface area and drug release. Dissolution rate formulation **(S2, S6, S10 & S15 with 9.98mm diameter)**was observed to be higher than formulation **(S3, S7, S11, S15 with 7.98mm diameter)**. This was due to the fact that **(S3, S7, S11, S15 with 7.98mm diameter)**implants had smaller surface area exposed to dissolution medium compared to **(S2, S6, S10 & S15 with 9.98mm diameter)**implant. From the above inferences**(Inference no. 1,2,3& 4)**it can be concluded that, "all theformulations give significantly different zero order release constants when evaluated by two different methods i.e., rotary flask and vial methods. The two methods used in the present investigation differ in two parameters: volume of dissolution medium and agitation speed. Hence as observed from **Table & 9**there was more release of drug from all formulation by rotary flask method. The higher drug release could be attributed to the agitation used in the Rotary Flask method and more amount of dissolution medium. In the Rotary Flask Method as the hydrodynamics are increased, there is decrease in diffusional distance and, hence, an increase in dissolution rate. These findings are important for optimization of clinically effective formulation.

**Table 1:** Solubility of disulfiram in different solvents

| Sr. No. | Solvent | Solubility (mg/ml) |
|---|---|---|
| 1 | Methanol | 33.05 |
| 2 | Water | 0.2 – 0.3 |
| 3 | Acetone | 119.37 |
| 4 | 0.1 M Phosphate buffer pH 7.4 | 0.25 – 0.35 |
| 5 | Tween – 80 | More than 125 mg |

**Table 2:** Calibration Curve Result

| Eq. of Line | Y= 0.0317X-0.0454 |
|---|---|
| $R^2$ | 0.9961 |

**Table 3:** Formulations of Disulfiram Implant

| Sr. No. | Formulation Code | Active ingredients (mg) | Diameter (mm) | Diameter (mm) | Compression force (lb/cm$^2$) |
|---|---|---|---|---|---|
| 1 | S2 | 200 | - | 9.98 | 550 |
| 2 | S6 | 200 | - | 9.98 | 1100 |
| 3 | S10 | 200 | - | 9.98 | 1650 |
| 4 | S14 | 200 | - | 9.98 | 2200 |
| 5 | S3 | 200 | 7.98 | - | 550 |
| 6 | S7 | 200 | 7.98 | - | 1100 |
| 7 | S11 | 200 | 7.98 | - | 1650 |
| 8 | S15 | 200 | 7.98 | - | 2200 |

**Table 4:** Evaluation of different formulation of Disulfiram

| | Formulation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Parameter** | **S2** | **S6** | **S10** | **S14** | **S3** | **S7** | **S11** | **S15** |
| Diameter (mm) | 9.98 (±0.094) | 9.98 (±0.089) | 9.98 (±0.670) | 9.98 (±0.044) | 7.98 (±0.230) | 7.98 (±0.361) | 7.98 (±0.610) | 7.98 (±0.156) |
| Thickness (mm) | 3.338 (±0.086) | 3.336 (±0.092) | 3.338 (±0.076) | 3.335 (±0.036) | 4.18 (±0.110) | 4.20 (±0369) | 4.15 (±0.236) | 4.12 (±0.338) |
| Hardness (Kg/cm$^2$) | 3.337 (±0.096) | 3.521 (±0.123) | 3.777 (±0.066) | 3.890 (±0.013) | 4.651 (±0.033) | 4.712 (±0.781) | 4.810 (±0.328) | 4.886 (±0.391) |
| Deviation in weight variation | 2.241 (+0.251) | 2.297 (+0.320) | 2.105 (+0.050) | 2.231 (±0.067) | 2.016 (±0.981) | 2.118 (±0.077) | 2.181 (±0.268) | 2.111 (±0336) |
| Drug content | 95.40 (+0.029) | 93.69(+0.055) | 95.15 (+0.038) | 97.21 (±0.071) | 95.91 (±0.086) | 96.23 (±0.021) | 94.69 (±0.037) | 95.22 (±0.047) |

**Table 5**: In vitro-release methods at a glance & Experimental design for in vitro drug release

| Parameter | Vial Method | R.F. Method |
|---|---|---|
| Quantity of phosphate buffer | 10.0ml | 100 ml |
| pH | 7.4 | 7.4 |
| Agitation speed | Shaken 5 min. at sampling | 25 R.P.M. |
| Temperature | $37^0$C + 0.5 $^0$C | $37^0$C + 0.5 $^0$C |
| Formulation | All | All |
| Time in Days | 100 to 200 | 100 to 200 |

**Table 6:** Mean (+ SEM) Cumulative percent of drug released by Vial method (n=3) from formulation (S2, S6, S10, S14 & S3, S7, S11, S15)

| | % Cumulative Release | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Time in days** | **S2** | **S6** | **S10** | **S14** | **S3** | **S7** | **S11** | **S15** |
| 10 | 13.78 (±1.03) | 12.97 (±1.56) | 10.65 (±1.22) | 9.36 (±0.78) | 12.88 (±1.11) | 11.63 (±1.33) | 9.12 (±1.07) | 8.7 (±1.46) |
| 20 | 19.96 (±0.87) | 17.36 (±1.23) | 16.63 (±1.63) | 15.87 (±1.29) | 16.94 (±0.98) | 15.82 (±1.46) | 14.32 (±1.62) | 13.56 (±1.08) |
| 30 | 26.78 (±1.23) | 24.32 (±1.46) | 23.35 (±1.45) | 21.98 (±1.63) | 23.01 (±1.43) | 21.1 (±1.68) | 19.21 (±0.63) | 18.23 (±1.27) |
| 40 | 36.65 (±0.39) | 30.89 (±1.57) | 30.21 (±1.39) | 28.65 (±1.42) | 31.87 (±0.96) | 29.46 (±1.54) | 26.33 (±1.49) | 24.87 (±1.18) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 50 | 48.79 (±1.21) | 38.12 (±0.98) | 37.63 (±1.52) | 35.81 (±1.36) | 40.21 (±0.77) | 36.57 (±1.32) | 33.45 (±1.53) | 32.12 (±1.36) |
| 60 | 56.01 (±0.67) | 47.23 (±1.25) | 44.96 (±1.16) | 42.63 (±1.55) | 48.08 (±1.32) | 45.94 (±1.71) | 40.61 (±1.37) | 38.32 (±1.12) |
| 70 | 65.95 (±0.96) | 55.1 (±1.64) | 50.77 (±1.26) | 48.91 (±1.28) | 58.98 (±1.22) | 52.56 (±0.69) | 46.18 (±1.22) | 44.74 (±1.43) |
| 80 | 74.86 (±1.32) | 61.39 (±1.37) | 57.81 (±1.44) | 55.23 (±1.30) | 65.6 (±1.05) | 58.05 (±0.94) | 51.93 (±1.74) | 50.07 (±1.37) |
| 90 | 98.68 (±0.77) | 72.77 (±0.63) | 63.69 (±0.67) | 61.64 (±0.79) | 75.97 (±1.36) | 65.36 (±0.73) | 57.63 (±1.23) | 55.65 (±1.66) |
| 100 | | 85.29 (±0.83) | 71.28 (±1.23) | 68.92 (±0.88) | 97.28 (±0.67) | 74.92 (±0.88) | 65.32 (±1.37) | 63.28 (±1.51) |
| 110 | | 98.19 (±1.09) | 83.91 (±1.62) | 81.54 (±1.35) | | 86.63 (±1.29) | 74.31 (±0.95) | 71.69 (±1.61) |
| 120 | | | 96.37 (±0.77) | 94.67 (±1.49) | | 97.27 (±1.36) | 86.13 (±1.38) | 83.18 (±1.28) |
| 130 | | | | | | | 97.63 (±1.12) | 95.71 (±0.86) |

**Table 7:** Mean (+ SEM) Cumulative percent of drug released by R.F. method (n=3) from formulation (S2, S6, S10, S14 & S3, S7, S11, S15)

| **% Cumulative Releases** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Time in days** | **S2** | **S6** | **S10** | **S14** | **S3** | **S7** | **S11** | **S15** |
| 10 | 16.91 (±1.24) | 14.96 (±0.71) | 11.67 (±0.86) | 11.69 (±0.76) | 16.18 (±0.86) | 13.23 (±1.16) | 11.23 (±1.13) | 9.79 ±1.16) |
| 20 | 24.22 (±0.63) | 18.29 (±1.16) | 18.69 (±1.16) | 17.11 (±1.31) | 22.16 (±1.66) | 19.56 (±1.23) | 16.77 (±1.62) | 15.67 (±1.23) |
| 30 | 31.63 (±1.36) | 25.63 (±1.62) | 26.12 (±1.62) | 24.23 (±0.66) | 29.32 (±1.39) | 25.63 (±1.64) | 22.78 (±0.85) | 21.36 (±1.66) |
| 40 | 41.56 (±0.94) | 33.77 (±1.54) | 34.28 (±1.44) | 31.6 (±1.71) | 36.64 (±0.94) | 34.23 (±1.32) | 30.65 (±0.46) | 28.69 (±1.47) |
| 50 | 52.63 (±1.45) | 43.52 (±1.33) | 41.67 (±1.36) | 41.22 (±1.28) | 45.10 (±1.49) | 42.23 (±0.64) | 37.12 (±1.12) | 36.98 (±1.32) |
| 60 | 64.12 (±1.26) | 53.26 (±0.91) | 49.86 (±1.22) | 48.67 (±1.45) | 56.32 (±1.35) | 52.32 (±1.16) | 45.66 (±0.73) | 43.26 (±0.59) |
| 70 | 78.31 (±1.07) | 65.12 (±1.64) | 57.65 (±1.71) | 55.36 (±1.56) | 67.66 (±1.92) | 61.96 (±1.38) | 54.23 (±1.26) | 51.36 (±1.22) |
| 80 | 95.63 (±1.48) | 76.23 (±1.55) | 65.29 (±0.66) | 63.67 (±0.59) | 79.77 (±1.38) | 73.23 (±0.77) | 61.98 (±1.32) | 57.23 (±1.37) |
| 90 | | 87.96 (±0.63) | 73.26 (±1.24) | 71.36 (±0.79) | 96.65 (±0.93) | 85.11 (±1.11) | 69.12 (±1.09) | 62.77 (±1.68) |
| 100 | | 98.21 (±1.24) | 84.69 (±1.43) | 83.67 (±1.69) | | 96.67 (±1.36) | 77.27 (±1.66) | 73.63 (±1.31) |
| 110 | | | 96.56 (±1.18) | 97.27 (±1.09) | | | 86.91) (±1.53) | 84.32 (±1.28) |
| 120 | | | | | | | 97.81 (±1.28) | 96.21 (±1.19) |

**Table 8:** Dissolution kinetic treatment to formulation by Vial Method

| **Formulation Code** | **Equation of Line** | **Regression Coefficient** | **Release Rate Constant** |
|---|---|---|---|
| | **Zero order** | **Zero order** | **Zero order** |
| S2 | y = 0.9972x - 0.7302 | 0.9804 | 0.9972 |
| S6 | y = 0.8342x - 0.5808 | 0.9866 | 0.8342 |

| | | | |
|---|---|---|---|
| S10 | y = 0.7431x + 0.5866 | 0.9910 | 0.7431 |
| S14 | y = 0.7288x - 0.2485 | 0.9891 | 0.7288 |
| S5 | y = 0.8844x - 1.4205 | 0.9788 | 0.8844 |
| S7 | y = 0.7457x + 0.4891 | 0.9942 | 0.7457 |
| S11 | y = 0.7025x - 1.2234 | 0.9885 | 0.7025 |
| S15 | y = 0.6529x - 0.3703 | 0.9936 | 0.6529 |

**Table 9:** Dissolution kinetic treatment to formulation by R.F. Method

| **Formulation Code** | **Equation of Line** | **Regression Coefficient** | **Release Rate Constant** |
|---|---|---|---|
| | **Zero order** | **Zero order** | **Zero order** |
| S2 | y = 1.1125x + 0.4998 | 0.9858 | 1.1125 |
| S6 | y = 0.8342x - 0.5808 | 0.9866 | 0.8342 |
| S10 | y = 0.8306x + 0.9627 | 0.9959 | 0.8306 |
| S14 | y = 0.8299x - 0.1562 | 0.9921 | 0.8299 |
| S5 | y = 0.9891x + 0.4725 | 0.9828 | 0.9891 |
| S7 | y = 0.9297x - 0.6495 | 0.9914 | 0.9297 |
| S11 | y = 0.7835x + 0.0281 | 0.9966 | 0.7835 |
| S15 | y = 0.7568x - 0.697 | 0.9919 | 0.7568 |

**Table 10:** Values of zero order release rate constants by vial& R.F. method (S2& S3)

| **Method** ⬇ | **Formulation S2** | | | **Formulation S3** | | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **N** | **Mean** | **SD** | **N** |
| **Vial** | 0.9972 | 0.0045 | 3 | 0.8844 | 0.0039 | 3 |
| **R.F.** | 1.1125 | 0.0087 | 3 | 0.9891 | 0.0041 | 3 |

**Table 11:** ANOVA for effect of surface area & method on in vitro release of formulation S2&S3

| **ANOVA Table** | **SS** | **DF** | **MS** | **F (DFn, DFd)** | **P value** |
|---|---|---|---|---|---|
| **Interaction** | 0.002589 | 1 | 0.002589 | F (1, 8) =55.78 | P<0.0001 |
| **Row Factor (Method)** | 0.03630 | 1 | 0.03630 | F (1, 8) = 1135 | P<0.0001 |
| **Column Factor (Surface Area)** | 0.04184 | 1 | 0.04184 | F (1, 8) = 1308 | P<0.0001 |
| **Residual** | 0.0002559 | 8 | 3.199e-005 | | |

P (< 0.0001) value summary: Effect of surface area: significant Effect of Method: significant

**Tukey's multiple comparison test (S2 & S3)**

| | | |
|---|---|---|
| **1** | Compare cell means regardless of rows and columns | |
| **2** | Number of families | 1 |
| **3** | Number of comparisons per family | 6 |
| **4** | Alpha | 0.05 |

**Table 12:** Tukey's multiple comparison test (S2 & S3)

| **Tukey's Multiple comparisons test** | **Mean Diff.** | **95.00% CI of diff.** | **Below threshold?** | **Summary** | **Adjusted P Value** |
|---|---|---|---|---|---|
| VMS2 vs. VM S3 | 0.1128 | 0.09801 to 0.1276 | Yes | **** | <0.0001 |
| VMS2 vs. RFMS2 | -0.1153 | -0.1301 to -0.1005 | Yes | **** | <0.0001 |
| VMS2 vs. RFMS3 | -0.1371 | -0.006689 to0.02289 | No | **** | <0.0001 |
| VMS3 vs. RFMS2 | -0.2281 | -0.2429 | Yes | **** | <0.0001 |

| | | | | | |
|---|---|---|---|---|---|
| | | to -0.2133 | | | |
| VMS3 vs. RFMS3 | -0.1047 | -0.1195 to -0.08991 | Yes | **** | <0.0001 |
| RFM S2 vs. RFMS3 | 0.1234 | 0.1086 to 0.1382 | Yes | **** | <0.0001 |

**Table 13**: Values of zero order release rate constants by vial& R.F. method (S6& S7)

| **Method** ⬇ | **Formulation S6** | | | **Formulation S7** | | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **N** | **Mean** | **SD** | **N** |
| **Vial** | 0.8342 | 0.0067 | 3 | 0.7457 | 0.0071 | 3 |
| **R.F.** | 0.9594 | 0.0056 | 3 | 0.9297 | 0.0077 | 3 |

**Table 14:** ANOVA for effect of surface area & method on in vitro release of formulation S6&S7

| **ANOVA Table** | **SS** | **DF** | **MS** | **F (DFn, DFd)** | **P value** |
|---|---|---|---|---|---|
| **Interaction** | 0.002593 | 1 | 0.002593 | F (1, 8) = 55.78 | P<0.0001 |
| **Row Factor (Method)** | 0.07170 | 1 | 0.07170 | F (1, 8) = 1542 | P<0.0001 |
| **Column Factor (Surface Area)** | 0.01048 | 1 | 0.01048 | F (1, 8) = 225.4 | P<0.0001 |
| **Residual** | 0.0003719 | 8 | 4.649e-005 | | |

P (< 0.0001) value summary: Effect of surface area: significant Effect of Method: significant

**Tukey's multiple comparison test (S6 & S7)**

| | | |
|---|---|---|
| **1** | Compare cell means regardless of rows and columns | |
| **2** | Number of families | 1 |
| **3** | Number of comparisons per family | 6 |
| **4** | Alpha | 0.05 |

**Table 15**: Tukey's multiple comparison test (S6 & S7)

| **Tukey's Multiple comparisons test** | **Mean Diff.** | **95.00% CI of diff.** | **Below threshold?** | **Summary** | **Adjusted P Value** |
|---|---|---|---|---|---|
| VM S6 vs. VM S7 | 0.08850 | 0.07067 to 0.1063 | Yes | **** | <0.0001 |
| VM S6 vs. RFM S6 | -0.1252 | -0.1430 to -0.1074 | Yes | **** | <0.0001 |
| VM S6 vs. RFM S7 | -0.09550 | -0.1133 to -0.07767 | Yes | **** | <0.0001 |
| VM S7 vs. RFM S6 | -0.2137 | -0.2315 to -0.1959 | Yes | **** | <0.0001 |
| VM S7 vs. RFM S7 | -0.1840 | -0.2018 to -0.1662 | Yes | **** | <0.0001 |
| RFM S6 vs. RFM S7 | 0.02970 | 0.01187 to 0.04753 | Yes | **** | <0.0001 |

**Table 16:** Values of zero order release rate constants by vial& R.F. method (S10& S11)

| **Method** ⬇ | **Formulation S10** | | | **Formulation S11** | | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **N** | **Mean** | **SD** | **N** |
| **Vial** | 0.7431 | 0.0021 | 3 | 0.7025 | 0.001 | 3 |
| **R.F.** | 0.8306 | 0.0018 | 3 | 0.7835 | 0.0012 | 3 |

**Table 17:** ANOVA for effect of surface area & method on in vitro release of formulation S10&S11

| ANOVA Table | SS | DF | MS | F (DFn, DFd) | P value |
|---|---|---|---|---|---|
| **Interaction** | 3.169e-005 | 1 | 3.169e-005 | F (1, 8) = 12.56 | P=0.0076 |
| **Row Factor (Method)** | 0.02129 | 1 | 0.02129 | F (1, 8) = 8442 | P<0.0001 |
| **Column Factor (Surface Area)** | 0.005768 | 1 | 0.005768 | F (1, 8) = 2287 | P<0.0001 |
| **Residual** | 2.018e-005 | 8 | 2.523e-006 | | |

P (< 0.0001) value summary: Effect of surface area: significant Effect of Method: significant

**Tukey's multiple comparison test (S10 & S11)**

| | | |
|---|---|---|
| **1** | Compare cell means regardless of rows and columns | |
| **2** | Number of families | 1 |
| **3** | Number of comparisons per family | 6 |
| **4** | Alpha | 0.05 |

**Table 18:** Tukey's multiple comparison test (S10 & S11)

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| VM S10 vs. VM S11 | 0.04060 | 0.03645 to 0.04475 | Yes | **** | <0.0001 |
| VM S10 vs. RFM S10 | -0.08750 | -0.09165 to -0.08335 | Yes | **** | <0.0001 |
| VM S10 vs. RFM S11 | -0.04040 | -0.04455 to -0.03625 | Yes | **** | <0.0001 |
| VM S11 vs. RFM S10 | -0.1281 | -0.1323 to -0.1239 | Yes | **** | <0.0001 |
| VM S11 vs. RFM S11 | -0.08100 | -0.08515 to -0.07685 | Yes | **** | <0.0001 |
| RFM S10 vs. RFM S11 | 0.04710 | 0.04295 to 0.05125 | Yes | **** | <0.0001 |

**Table 19:** Values of zero order release rate constants by vial& R.F. method (S14&S15)

| Method ⬇ | Formulation S14 | | | Formulation S15 | | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **N** | **Mean** | **SD** | **N** |
| **Vial** | 0.7288 | 0.0029 | 3 | 0.6529 | 0.0031 | 3 |
| **R.F.** | 0.8292 | 0.0038 | 3 | 0.7568 | 0.0036 | 3 |

**Table 20:** ANOVA for effect of surface area & method on in vitro release of formulation S14& S15

| ANOVA Table | SS | DF | MS | F (DFn, DFd) | P value |
|---|---|---|---|---|---|
| **Interaction** | 9.187e-006 | 1 | 9.187e-006 | F (1, 8) =0.8091 | P=0.3947 |
| **Row Factor (Method)** | 0.03130 | 1 | 0.03130 | F (1, 8) = 2757 | P<0.0001 |
| **Column Factor (Surface Area)** | 0.01649 | 1 | 0.01649 | F (1, 8) = 1453 | P<0.0001 |
| **Residual** | 9.084e-005 | 8 | 1.136e-005 | | |

P (< 0.0001) value summary: Effect of surface area: significant Effect of Method: significant

**Tukey's multiple comparison test (S14 & S15)**

| | | |
|---|---|---|
| **1** | Compare cell means regardless of rows and columns | |
| **2** | Number of families | 1 |
| **3** | Number of comparisons per family | 6 |
| **4** | Alpha | 0.05 |

**Table 21:** Tukey's multiple comparison test (S14 & S15)

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| VM S14 vs. VM S15 | 0.07590 | 0.06709 to 0.08471 | Yes | **** | <0.0001 |
| VM S14 vs. RFM S14 | -0.1004 | -0.1092 to -0.09159 | Yes | **** | <0.0001 |
| VM S14 vs. RFM S15 | -0.02800 | -0.03681 to -0.01919 | Yes | **** | <0.0001 |
| VM S15 vs. RFM S14 | -0.1763 | -0.1851 to -0.1675 | Yes | **** | <0.0001 |
| VM S15 vs. RFM S15 | -0.1039 | -0.1127 to -0.09509 | Yes | **** | <0.0001 |
| RFM S14 vs. RFM S15 | 0.07240 | 0.06359 to 0.08121 | Yes | **** | <0.0001 |

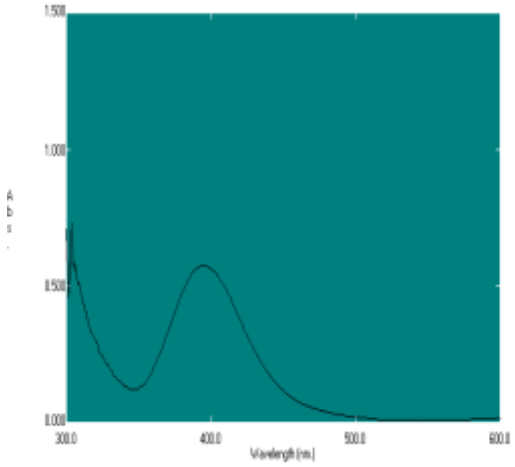

**Fig 1:** UV Spectrum of Disulfiram in 0.1%w/v cupric chloride solution

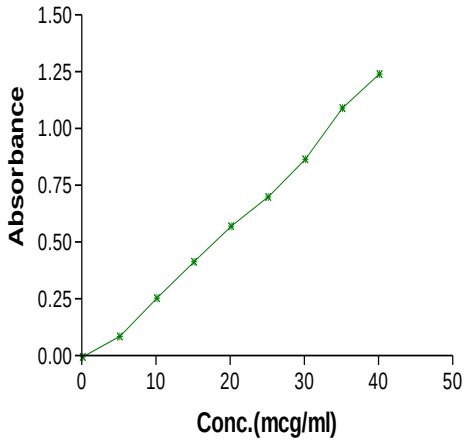


**Fig 2**: Beer - Lambert's plot of Disulfiram

**f) FTIR analysis of Disulfiram drug, PLGA polymer and combined Drug and polymer: -**

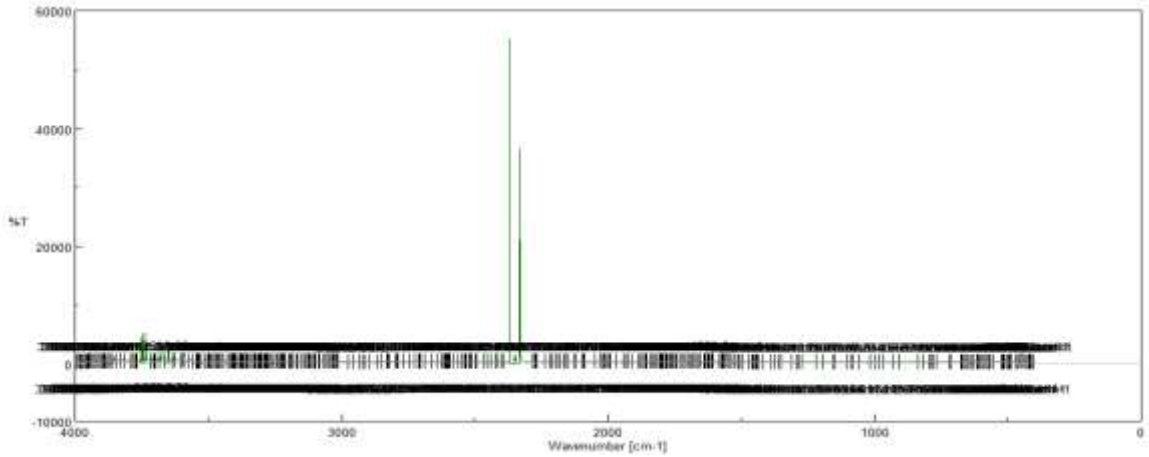

**Fig 3:** FTIR of Disulfiram

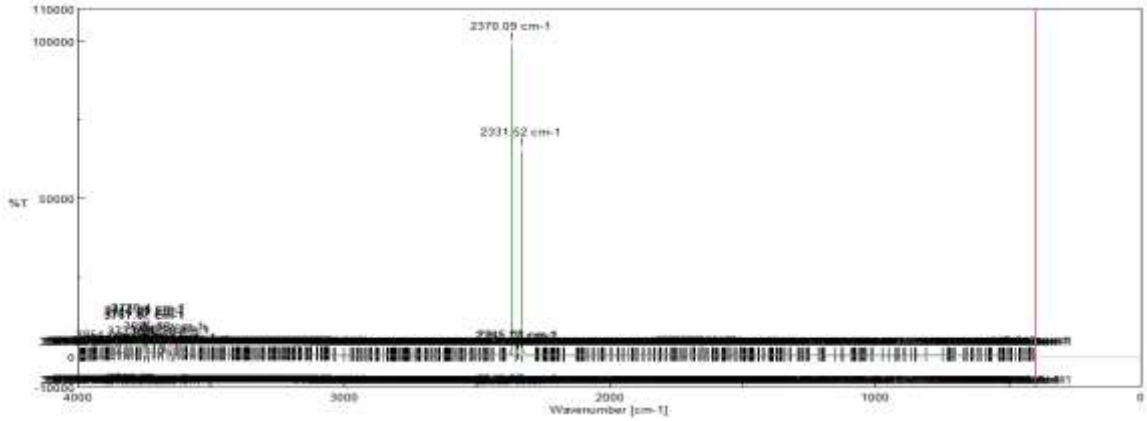


**Fig 4:** FTIR of PLGA

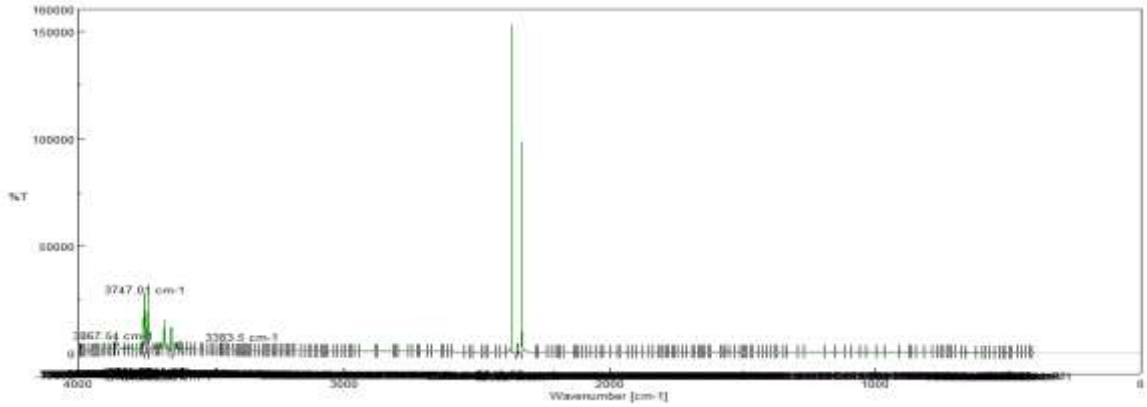


**Fig 5:** FTIR of Disulfiram and PLGA effect of surface area (vial method)

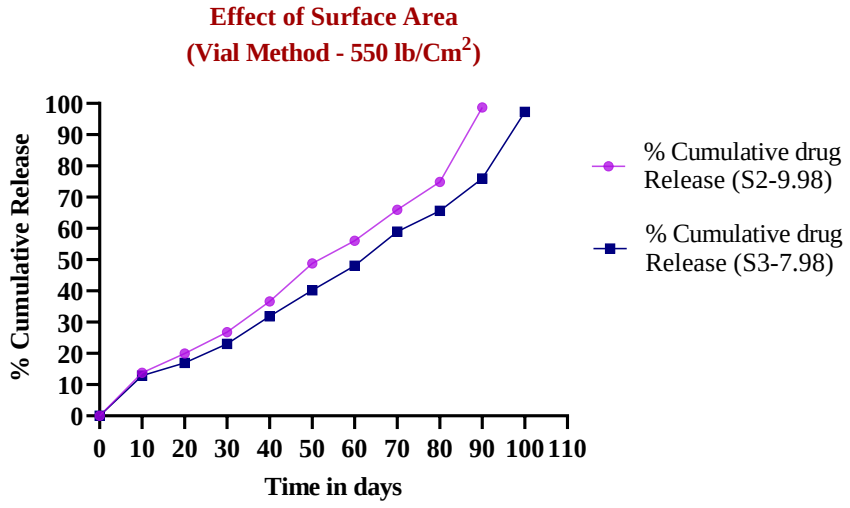


**Figure 6:** % Cumulative release of drug formulation S2 & S3 by Vial Method

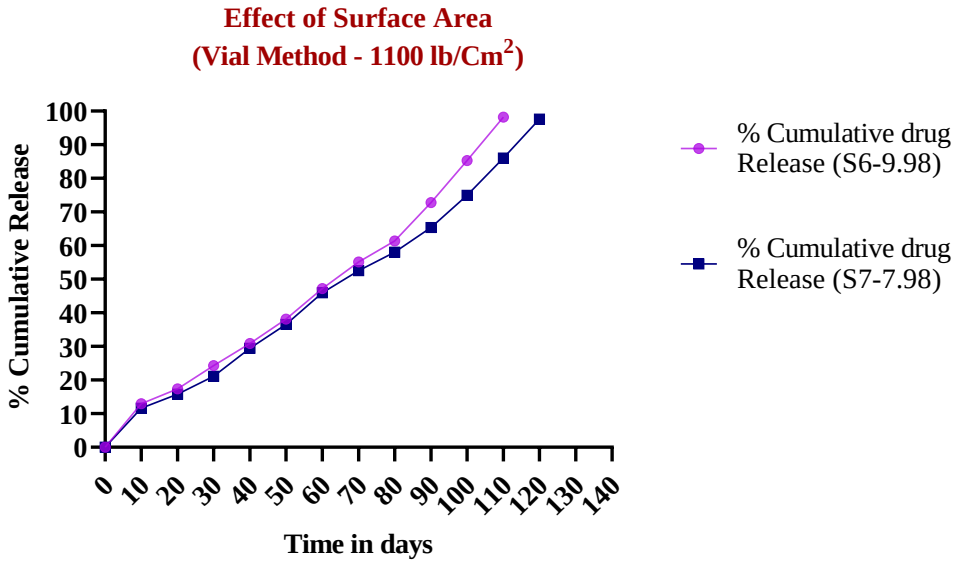


**Figure 7:** % Cumulative release of drug formulation S6 & S7 by Vial Method

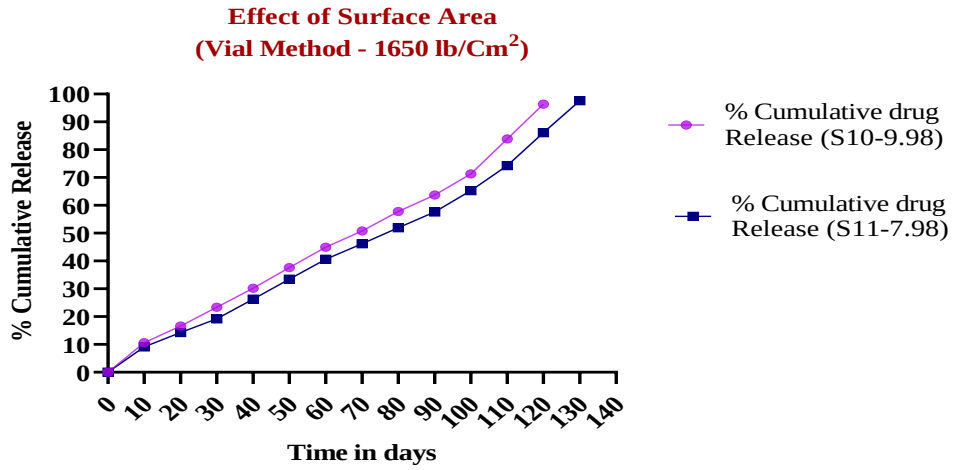


**Figure 8**: % Cumulative release of drug formulation S10 & S11 by Vial Method

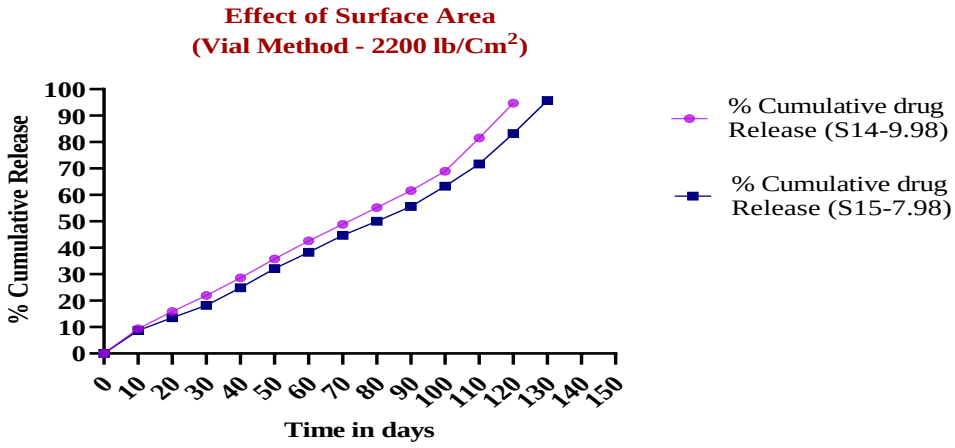


**Figure 9:** % Cumulative release of drug formulation S14 & S15 by Vial Method

**EFFECT OF SURFACE AREA (R.F. METHOD)**

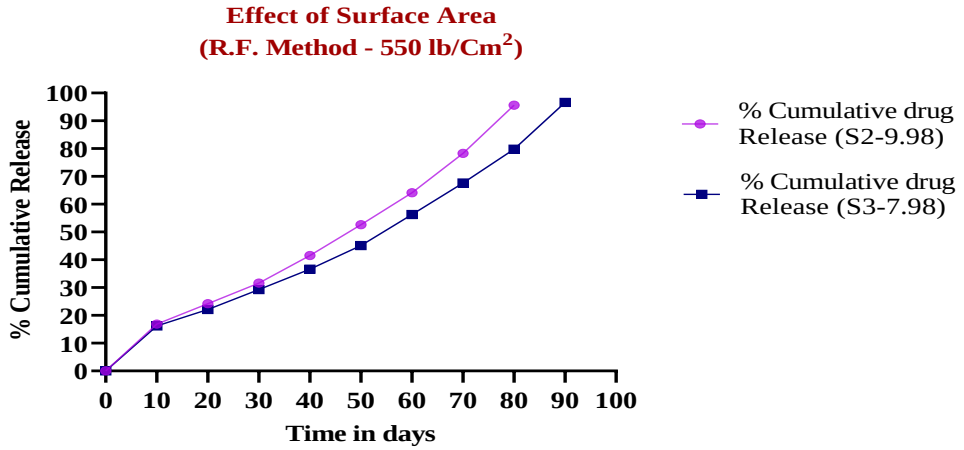


**Figure 10:** % Cumulative release of drug formulation S2 & S3 by R.F. Method

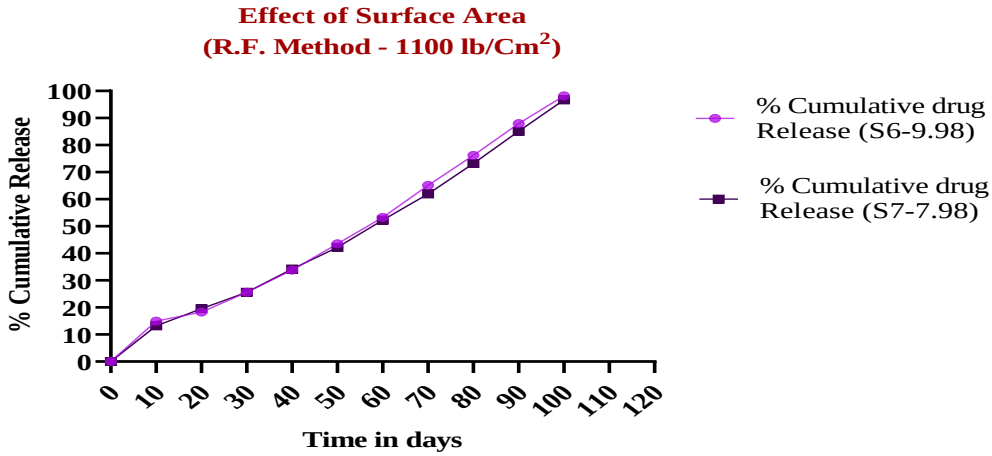


**Figure 11:** % Cumulative release of drug formulation S6 & S7 by R.F. Method

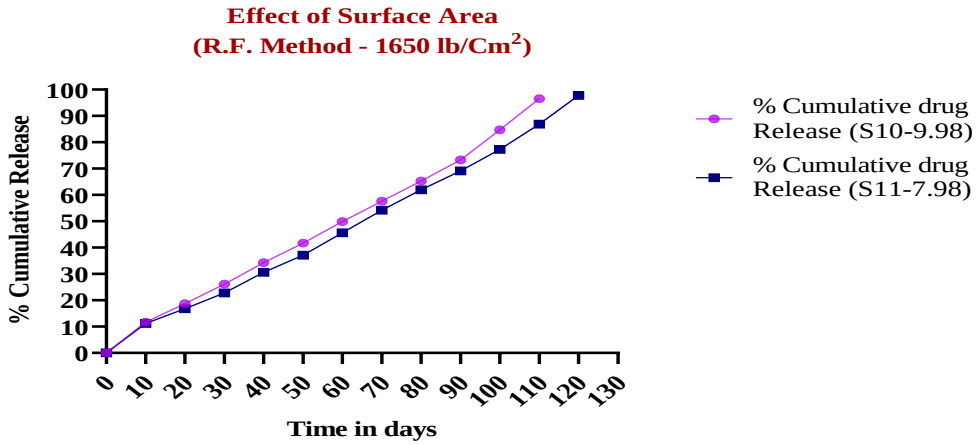


**Figure 12:** % Cumulative release of drug formulation S10 & S11 by R.F. Method

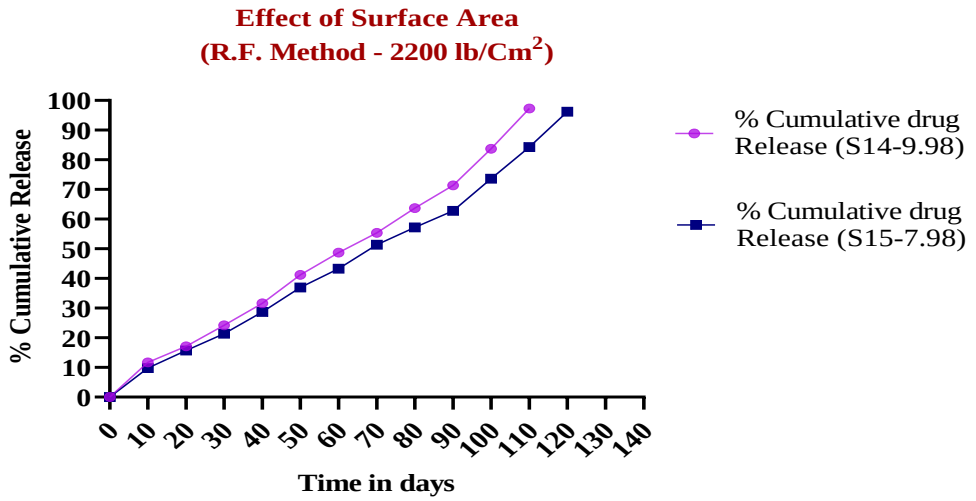


**Figure 13:** % Cumulative release of drug formulation S14 & S15 by R.F. Method

## CONCLUSION

The proposed work concluded that disulfiram can be directly compressed to prepare implantable pellets. From the statisticalInferences it was concluded that surface area had marked effect on in vitro release pattern of disulfiram implant. It was also observed that direct relationship exists between surface area and drug release i.e., increase in surface area increases the release rate. The release kinetic mechanism from all the formulation was found to be zero order. Both the methods of in vitro dissolution testing are found significantly different for all the formulations prepared on laboratory I.R. Press.

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