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Extraction of Chlorophyll from Alfalfa Plant

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Abstract:-
The extraction process of chlorophyll from dehydrated and pulverized alfalfa plant were studied by percolation method. Two solvent systems were used for the extraction namely; Ethanol-water and Hexane-Toluene systems . The effect of circulation rate, solvent concentration, and solvent volume to solid weight ratio were studied. In both ethanol water, and Hexane-Toluene systems it appears that solvent concentration is the most effective variable.

Keywords: Chlorophyll, Extraction, Percolation, alfalfa

1.Introduction

Chlorophyll, so defined, is a mixture of green pigments, which is always combined by other pigments, principally those of the carotenoid group [1]. Alfalfa is usually used as a green plant source for the extraction of chlorophyll because of its availability along all seasons of the year and because of its low price. Chlorophyll is relatively labile and during isolation it is necessary to protect it from degradation. As a general precaution, it is advantageous to work in dim light and low temperature to avoid pigment loss[2].

For extraction of high quality chlorophyll, there are many laboratory methods [3-10]. The most popular applied process is the extraction of chlorophyll by hexane or ethanol where both batch (percolation) and continuous processes (CSTR) were used [11,12].

No data appeared in literature for the evaluation of process parameters for the extraction of chlorophyll. Hence the purpose of this study is to investigate the process parameters for the extraction of chlorophyll from dehydrated alfalfa plant using percolation method. These parameters are namely; circulation rate of solvent, type and concentration of solvent, and volume of solvent to solid weight ratio. Different solvent systems were used for the extraction, namely, ethanol-water, and hexane-toluene blend.

2.Experimental Work Material

The alfalfa used for extraction was first dried in an oven at 45-50^{0}C for 1-2 hours[11]. Then the dried alfalfa was finely ground in a ball mill and then screened in a 50-mesh screen[11]. The -50 mesh size cut of alfalfa was used in the extraction experiments. Ethanol (95%, near isotropic concentration obtained from atmospheric distillation) used in ethanol-water system was supplied by General Company For Drinking and Food Industry. Hexane is supplied by Tagi gas plant while Toluene (99.9%) was supplied by Arabian Company for Detergents Chemicals.

3. Description of Equipment

Extraction experiments were carried out in QVF glass rig (Fig. 1) [13]. The extractor is a QVF cylindrical vessel of 21 cm in diameter and 58 cm height. In the extractor a cloth pocket is introduced filled with alfalfa. Liquid is distributed on the alfalfa pocket by a porous glass distributor. Solvents were circulated from a 20 liter QVF spherical vessel. A centrifugal pump(Q=2160 l/h, Head = 3m) were used for solvent extraction. A QVF Rota meter (0-400 l/h) was used to control the volumetric flow rate of solvent. The batch distillation system for the recovery of chlorophyll and solvents consists of QVF cylindrical vessel, QVF condenser with double coil of 8 cm in diameter and 50 cm high. A 148 kW electrical heater (Stabilag, England) was used in batch distillation. A digital temperature indicator controller was used for controlling temperature in batch distillation within 0 – 400 ^{0}C. A chiller (MGW LAUDA) was used for cooling of water necessary for the condensation of solvent vapors in the condenser. A ball mill (Siemens) was used for grinding dehydrated alfalfa meal.

4. Experimental Procedure

For the extraction system, the cloth pocket is filled with 1 kg of pulverized dehydrated alfalfa meal and then placed in the extraction cylindrical vessel. The spherical circulating vessel is filled with a certain volume of solvent. Before starting the experiments the solvent is heated to 35^{0}C and kept constant at this temperature throughout the experiments using a temperature controller in order to compare the different operation conditions at constant temperature . Then the circulating pump was switched on to circulate the solvent through the extractor and the spherical vessel. A needle valve was used for adjusting the volumetric flow rates of solvent at the desired value as indicated on the Rotameter. The circulation of solvent was continued for a maximum duration of 3 hours. Every fifteen minutes, a sample was taken for the solvent by a pipette. The concentration of chlorophyll in the sample was measured by atomic absorption spectrophotometer at the university of technology by measuring the ratio of absorbencies at 654 nm (A645) and 663 nm (A663). The concentration can then be calculated using the following equation[17]:

Total Chlorophyll (mg/L) = 20.2 A645 + 8.02 A663 ------------------ (1)

When the concentration of chlorophyll remains constant, the extraction experiment was stopped.

Batch distillation is used for the concentration of the product and for the recovery of solvent. The temperature of distillation of solvents must not affect the color of the solute (chlorophyll). This temperature was 90^{0}C for ethanol-water system and 80 ^{0}C for hexane-toluene system. This process for the separation of concentrated chlorophyll and recovery of the solvent were proceeded for 5-6 hours.

5. Results & Conclusion

The effect of operating variables on the extraction rate of chlorophyll for

ethanol-water and hexane-toluene systems from alfalfa were studied in this work.
The percentage of extracted chlorophyll was calculated using the following relationship :

$$\% \text{ extracted chlorophyll} = \frac{\text{Amount of chlorophyll dissolved}}{\text{Total amount of chlorophyll in Dehydrated alfalfa meal}}$$

6. The Effect of Circulated Rates of Solvent

both system (Figs. 2 and 3), the percentage of extracted chlorophyll increased with increasing circulation rates of solvent. The two main steps of mass transfer in the extraction process are diffusion of the fluid through solid and diffusion through film surrounding solid particles. Since solid particles are pulverized, the resistance to mass transfer by molecular diffusion of the solute from solid internal pores to the film surrounding solid particles will be smaller than the solute mass transfer across the film surrounding the particles to the solvent bulk. Hence, the expected rate controlling step is the diffusion of solute through film surrounding solid particles to the solvent bulk.

It is well known that extraction efficiency increased with increasing velocity of solvent. This phenomena is applicable for laminar flow ($131 < Re < 431$) , since in turbulent flow increasing of velocity of solvent have little effect on extraction rate [14]. In our case, the flow inside extractor is streamline (laminar), so the effect of velocity is noticed.

7. The Effect of Solvent Concentration

In ethanol-water system (Fig. 4), it can be noticed that the percentage of extracted chlorophyll increased as concentration of ethanol increased. This behavior can be explained as follows; higher concentration of ethanol means lower viscosity of solvent. This will lead to higher wetting of solids and hence increase extraction rates[16]. It also appeared in Fig. 3 that lower concentration of ethanol (50%) indicates lower extraction efficiency of chlorophyll. Hence, the minimum concentration used for the extraction of chlorophyll must be not less than 70%. This result is in agreement with the conclusion of Othmer [15].

The effect of blending of hexane and toluene on the percentage of chlorophyll extract is shown in Fig. 5. The percentage of extracted chlorophyll when using a pure hexane did not exceed 77%. At toluene concentration of less or equal to 21% by volume, the extraction efficiency is increased, while the extraction efficiency is decreased at toluene concentration greater than 21% [13]. This behavior can be explained as follows[9] ; the phytol group ($C_{20}H_{39}OH$) is a long-chain alcohol separated from the structure of chlorophyll and when the extraction process started, the phytol group is freely mobile in the liquid phase. This layer of phytol is impeded the non-polar solvent (hexane) to penetrate through the chlorophyll [9]. But when a certain volume of toluene (21%) is blended with hexane, a reaction occurs between toluene and phytol group which produced aromatic alcohol (benzyl alcohol). This aromatic is considered a more stronger solvent than pure hexane. On increasing the percentage of toluene above 21%, the physical properties of toluene as a solvent will be the controlling step, hence the extraction efficiency dropped.

8. The effect of solvent volume to solid weight ratio

As shown in Figs. 6,7, the percentage extracted chlorophyll increased with increasing volume of solvent for both system. This behavior could be explained as follows; as the volume of the solvent increased, the concentration difference (which is one of the most effective driving forces for mass transfer) will be increased and hence the extraction efficiency will be increased. But on increasing volume of solvent with respect to solid weight, the cost of solvent recovery will be increased with respect to volume of the distillation unit and the power applied.

9. Conclusion

1- The extraction rate of chlorophyll increased when; increasing the circulation rate of solvent in the two systems, increasing the concentration of ethanol, blending less than 21% of toluene in toluene-hexane system, and increasing solvent volume to solid weight ratio.
2- For ethanol water system, The effect of ethanol concentration on percentage of extracted chlorophyll was found to be most effective compared to circulation rate of solvent and volume of solvent to weight ratio. While the minimum concentration of ethanol used in the extraction must not be less than 70%.
3- In hexane-toluene system, the effect of hexane toluene blend is the most effective parameter on percentage of extracted chlorophyll compared to the effect of circulation rates and solvent volume to solid weight ratio. While the optimum concentration of blend was found to represent 21% toluene and 79% hexane.

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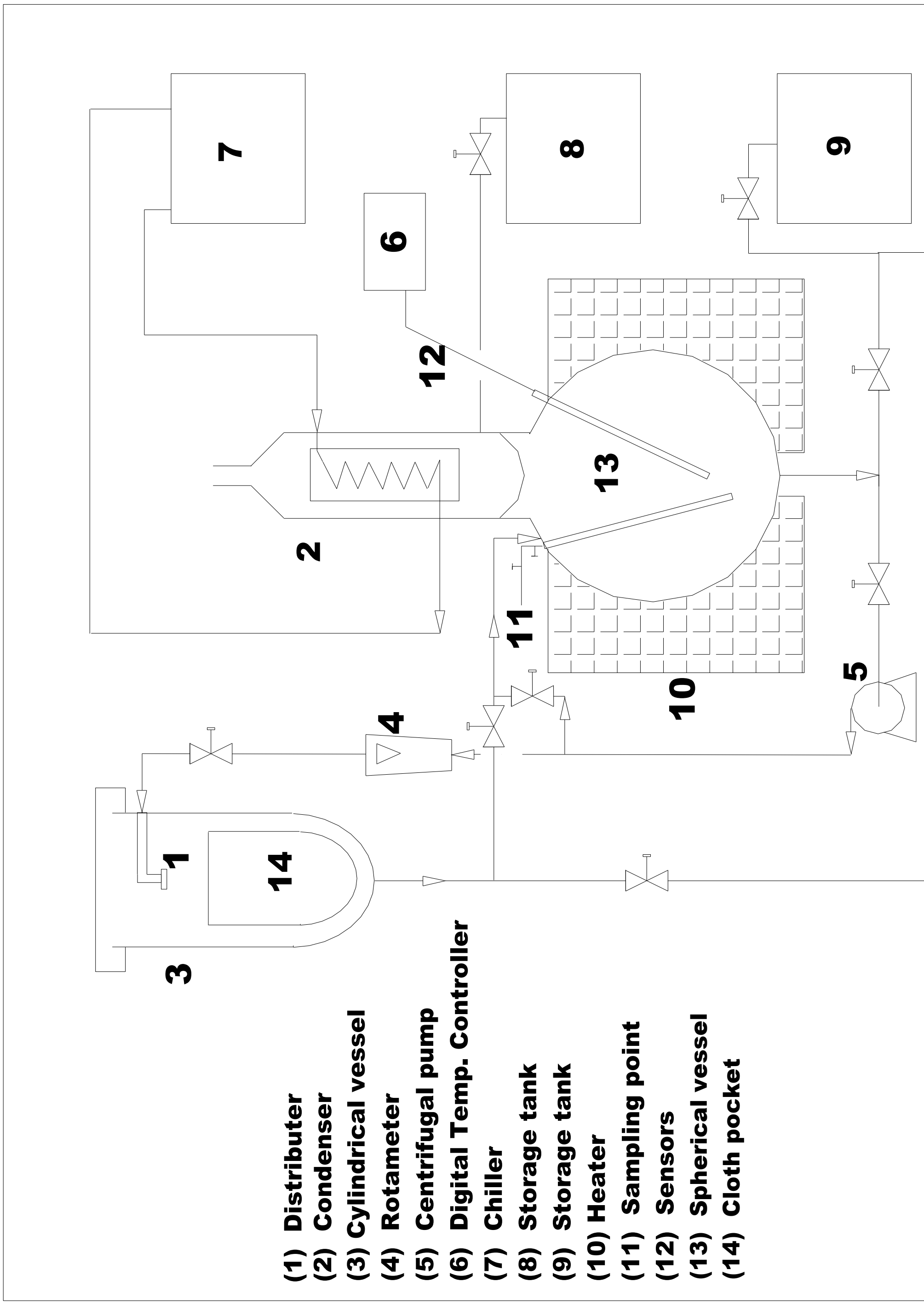
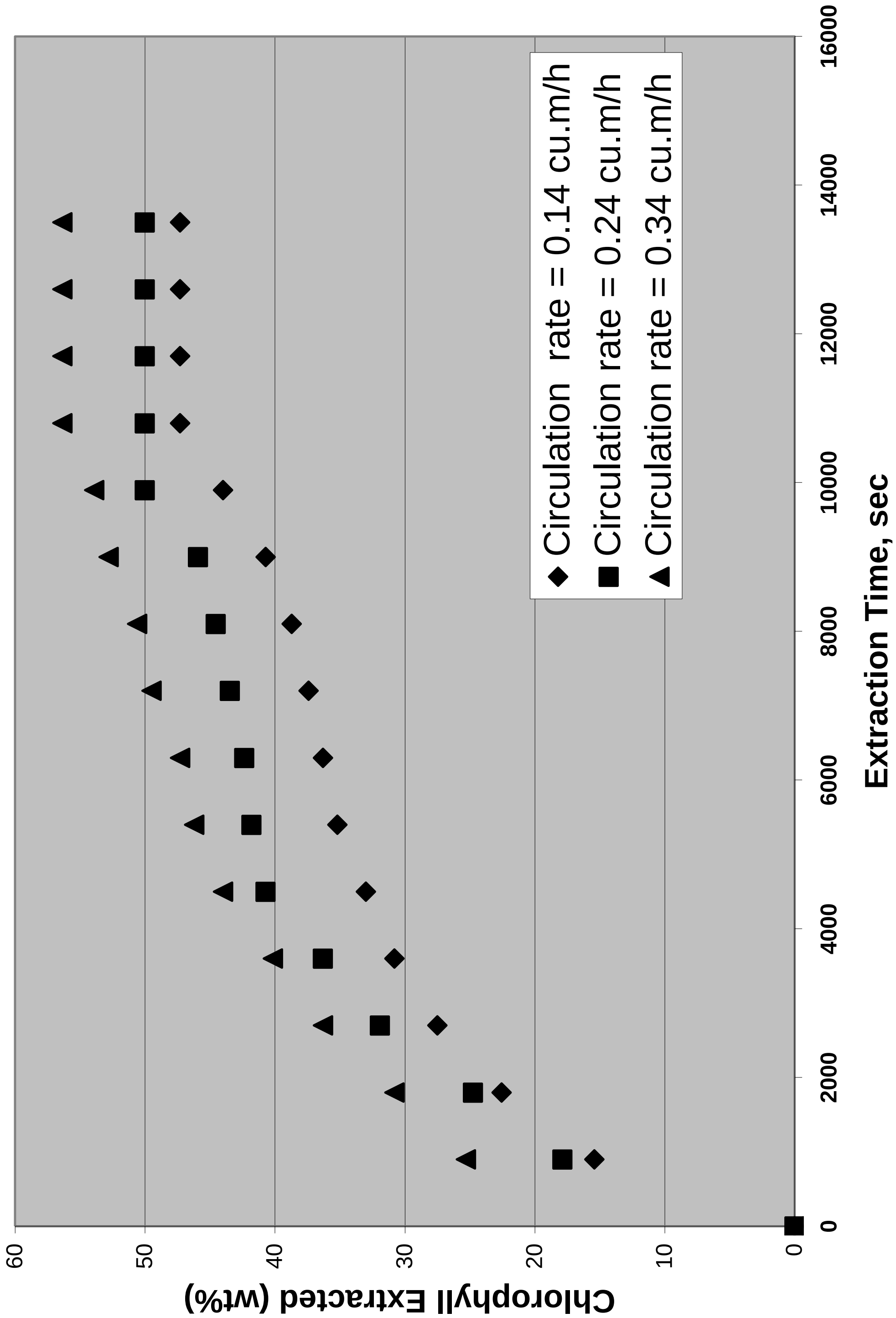
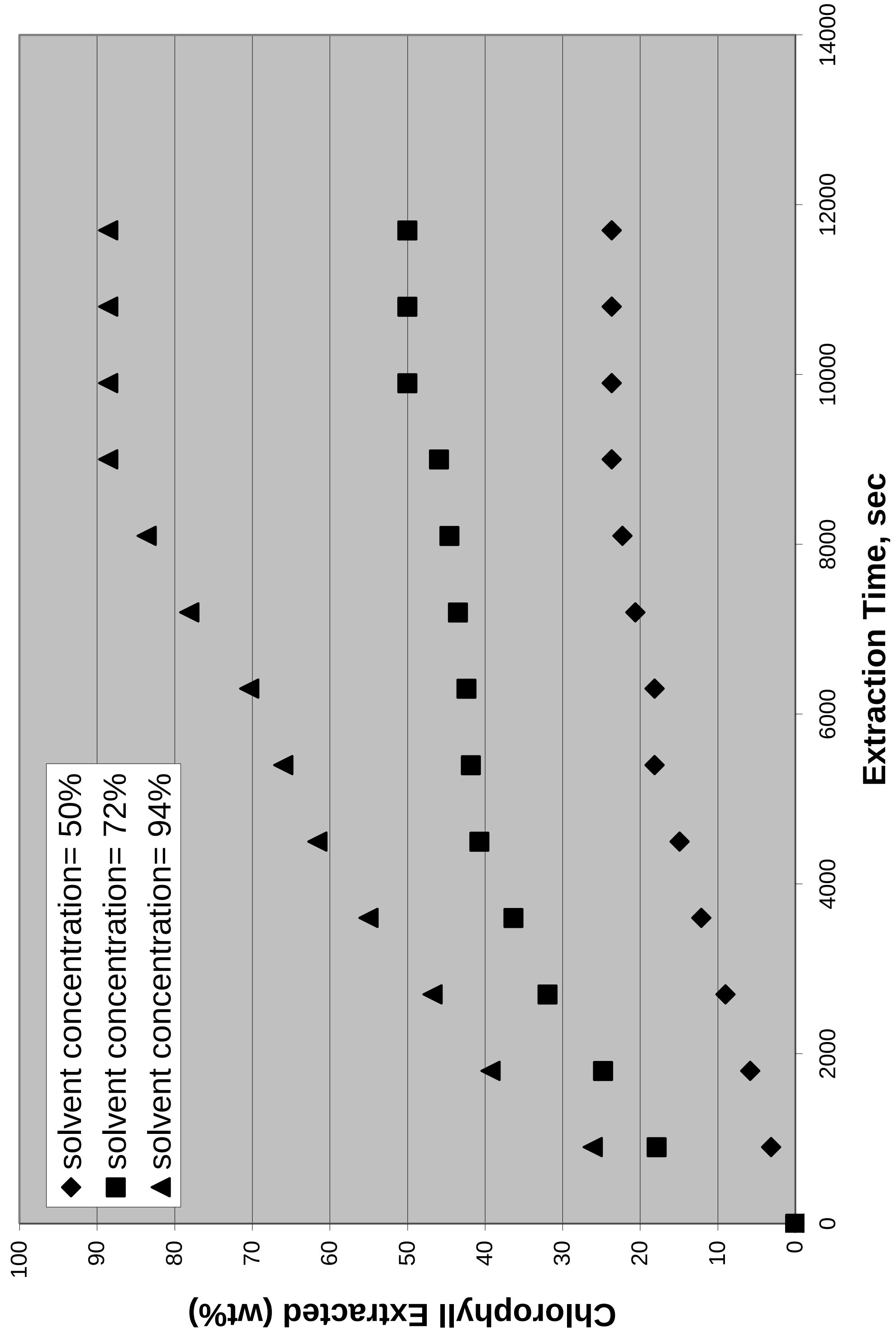


Figure (1) : General Flow Diagram of Experimental Rig.

Figure (2) : Effect of circulation Rates on Chlorophyll Extraction with 72% Ethanol (vol. of solvent/wt. of solids : 11/1).



Figure(3) : Effect of solvent concentration on chlorophyll extraction with ethanol solution at a circulation rate of 0.24 cu.m/h (vol. of solvent/wt. of solid : 11/1).



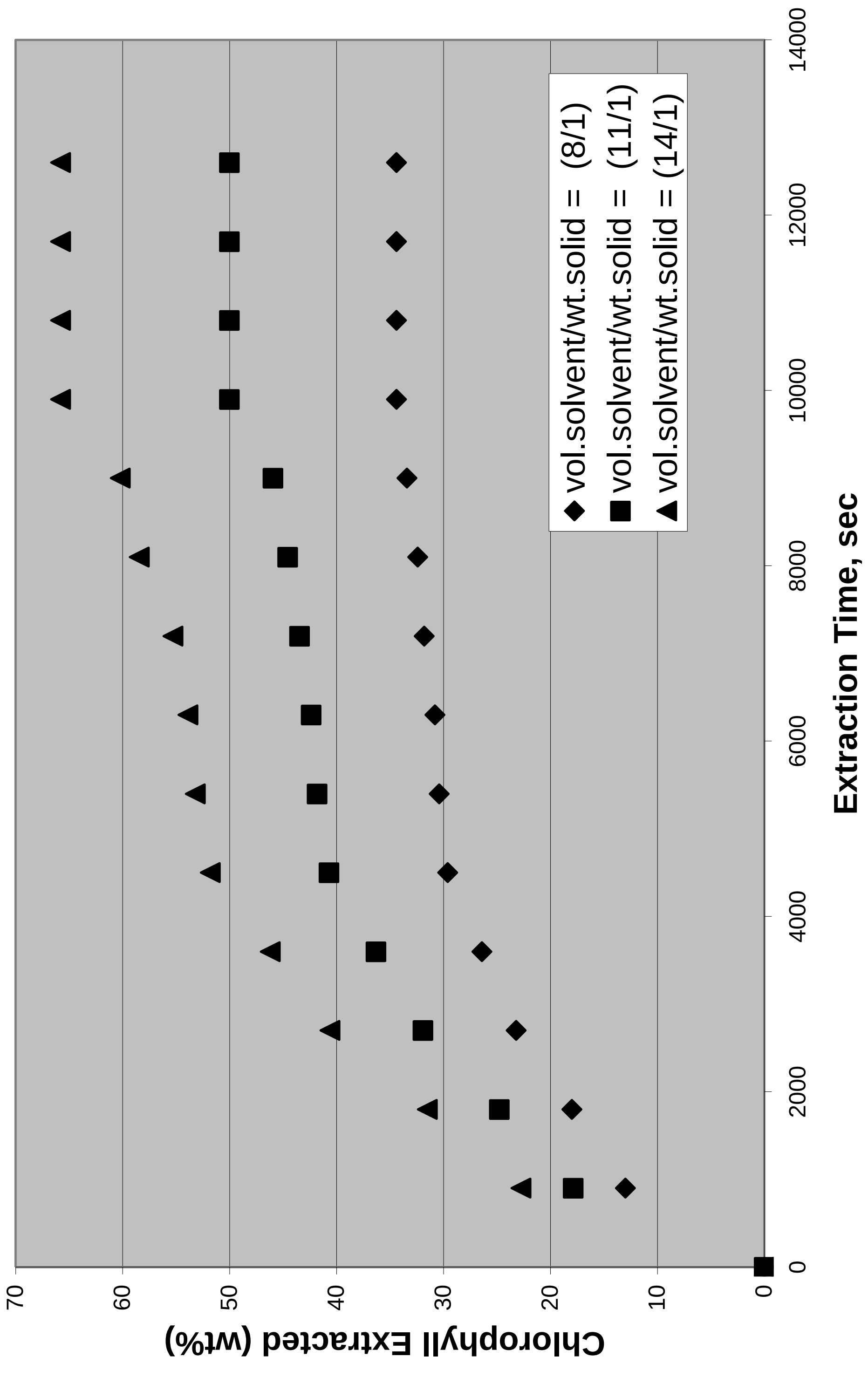


Figure (4) : Effect of Vol. of Solvent/Wt. of Solid Ratio on Chlorophyll Extraction with 72% Ethanol at A Circulation Rate of 0.24cu.m/h.

Figure (5) : Effect of Circulation Rate on Chlorophyll Extraction with Hexane-Toluene Blend (79% Hexane) (Vol. of Solvent/Wt. of Solid :11/1).

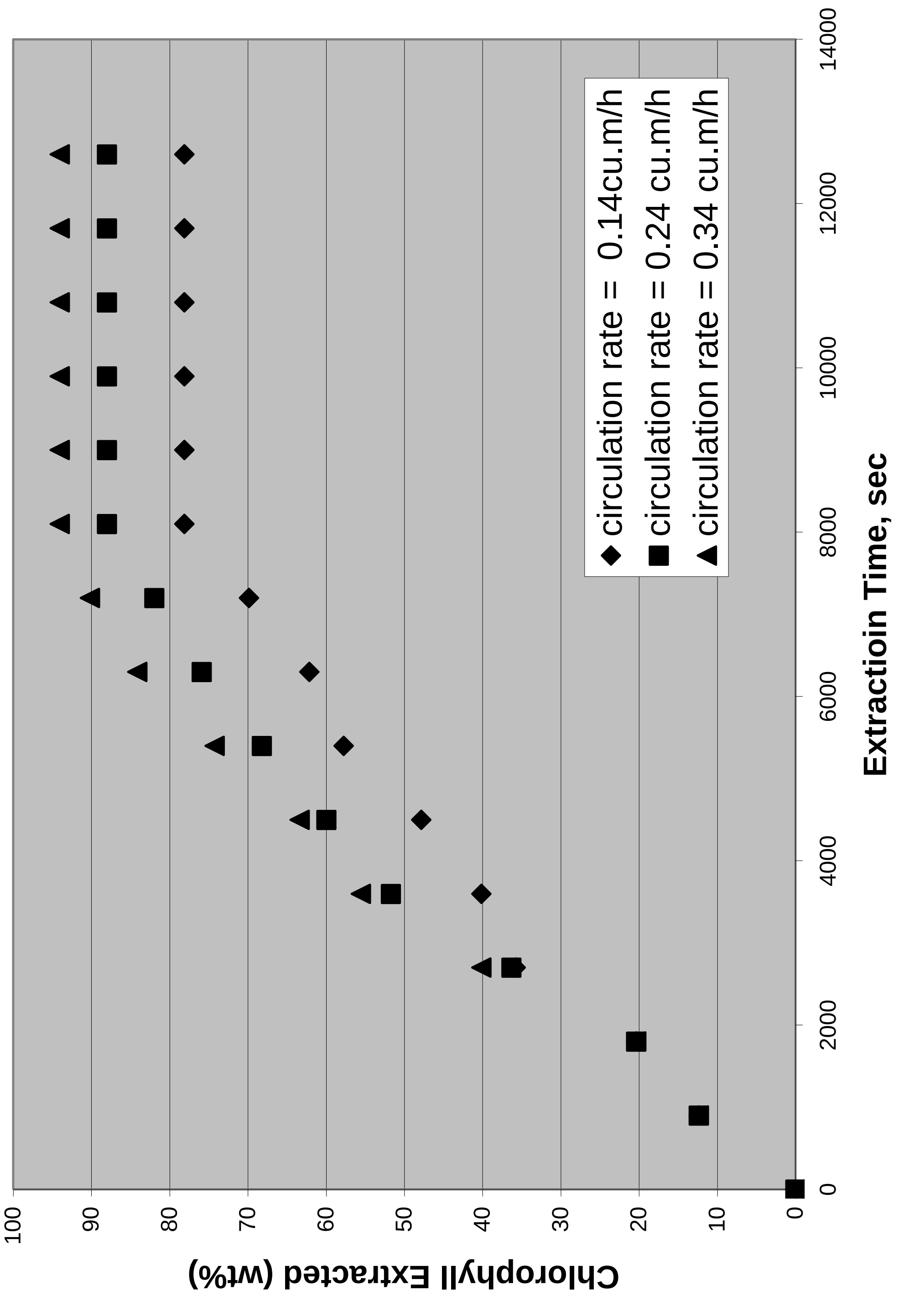


Figure (6) : Effect of Solvent Concentration on Chlorophyll Extraction with Hexane-Toluene Blend at A Circulation Rate of 0.24 cu.m/h.

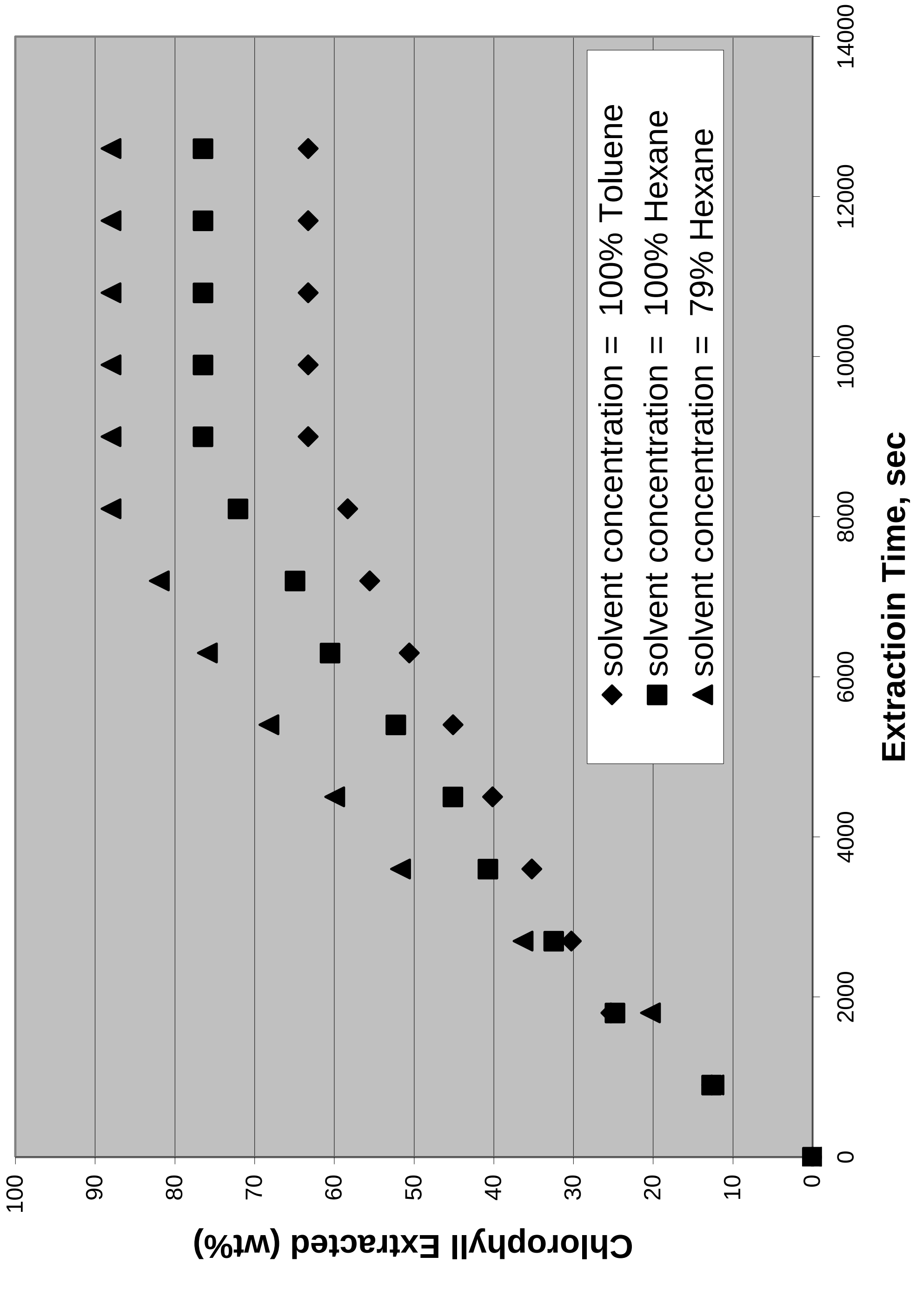
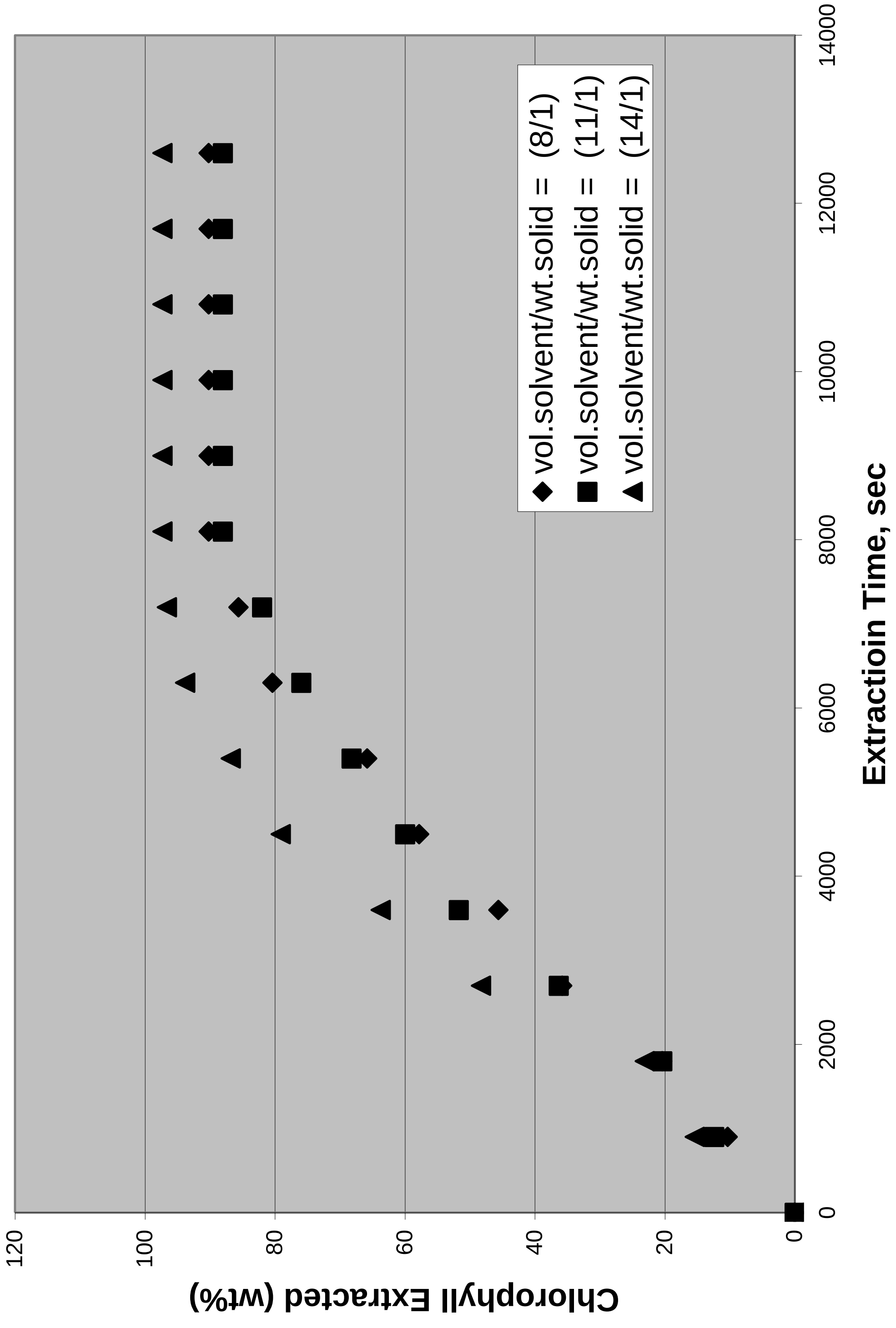


Figure (7) : Effect of Vol. of Solvent/Wt. of Solid Ratio on Chlorophyll Extraction with Hexane-Toluene Blend (79% Hexane) at A Circulation Rate of 0.24 cu.m/h.



أستخلاص الكلوروفيل من نبات الجت

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الخلاصة:

تم في هذا البحث دراسة عملية أستخلاص الكلوروفيل من الجت المجفف والمطحون بطريقة التغلغل. تم أستخدام نظامين من المذيبات للأستخلاص وهي منظومة أيثانول-ماء ومنظومة هكسان-تولوين . تم دراسة تأثير معدل التدوير، تركيز المذيب و نسبة حجم المذيب الى وزن المادة الصلبة المستخدمة . في كلا المنظومتين تم التوصل الى أن تركيز المذيب هو العنصر الأكثر تأثيرا.