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# Microwave-Assisted Preparation of Cross-Linked Gelatin-Paracetamol Matrices: Optimization using D-Optimal design

## Effects of microwave on release Paracetamol gelatin matrices

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**Abstract:**
**Objective:** The study was conducted to assess effect of microwave heating for preparation of paracetamol cross-linked gelatin matrices using DoE approach to explore effect of microwave irradiation time, concentration of cross linker and amount of sodium bicarbonate (salt) on paracetamol release which was also compared to matrices prepared using conventional heating.
**Material and Methods:** Twenty gel matrices were prepared with different microwave irradiation time, amounts of maize and sodium bicarbonate suggested by DoE software (Design Expert, DX®). % drug release, coefficient of variance (CV) in release and mean dissolution time (MDT) were properties in designed experimentation. It was found that target responses were dependent on microwave irradiation time, cross-linker concentration and salt concentration.
**Results:** Classical and microwave heating though did not demonstrate statistically significant difference in modifying % drug release from matrices. However, lower CV values were observed in case of microwave-assisted formulations compared to gel matrices prepared using classical heating. Thus, microwave heating produced lesser variations in drug release. Optimized gel matrices demonstrated that observed results of % drug release, CV and MDT were within prediction interval generated by DX®. Moreover, release mechanism of matrix formulations followed Peppas-Korsmeyer anomalous transport model.
**Conclusion:** Results suggested DoE-supported microwave-assisted approach could be applied to optimize critical factors for achieving drug release with less variation.
**Keywords:** Controlled release gel matrices, Paracetamol, Microwave heating, Classical heating, Design of Experiment

## Introduction

Controlling drug release from polymeric matrix is challenging in developing controlled release dosage forms due to different release patterns of drugs secondary to their physicochemical properties.[1, 2] The delay in drug release can be achieved by cross-linking of drug with polymeric matrices [3]. Chemical cross-linking is associated with residuals of chemical cross-linker, as issue which can be overcome by using biopolymers [4] for cross-linking with natural and non-toxic cross-linkers, such as maize due to their safety, biodegradability and biocompatibility. Literature cites use of microwave-assisted method for cross-linking and in developing dosage forms.[5, 6] Microwave emits electromagnetic radiations of frequency 300 GHz to 300 MHz. Most commercial microwave ovens produce microwave wavelength of 12.25 cm, equivalent to 2.45 GHz (6). Microwave heating can modify state of molecular interaction between polymer chains to control physicochemical properties and drug release [1]. Microwave energy is absorbed by material and converted into molecular kinetic energy and dissipated by molecule due to inertial, elastic and frictional forces of surroundings. Its specific heating causes dipolar polarization [7] and promotes cross-linking without use of harsh solvents [8] Carbohydrate polymers such as alginate, gelatin, and cellulose have been incorporated in controlled release drug delivery systems.[3, 4] Sodium alginate water soluble biopolymer which is cross-linked in presence of multivalent cations in aqueous media. This may form hydrogel upon cross-linking.[3] Calcium alginate-coated matrices significantly reduced drug release by main matrix structure.[1] Gelatin natural polymer is rarely used alone because of its low intensity and high brittleness unless it is modified by several methods such as cross-linking, grafting, and blending. Traditional experimentation for optimizing multiple factors of formulation is time-consuming and does not reveal factor interactions which may be synergistic or antagonistic. Conversely, design of experiment (DoE) determines relationship between factors and response finds factor interaction and facilitates developing an optimized formulation with lesser time, cost and material.[2, 9] In microwave-assisted cross-linking, factors expected to affect may include microwave power, microwave exposure time and temperature. Concentration of macromolecule, cross-linker, salt, and nature of drug may be other factors in designing gel formulation.

The aim of current study was to assess effect of microwave heating, in presence of cross-linker and salt, on cross-linking and thus, on drug release characteristics from gelatin matrices to optimize above conditions for release of drug using DoE approach. Paracetamol, a water soluble drug was used as model drug because of its easy availability and lack of risks in handling due to being over the counter drug, which makes rigorous testing of this drug possible in a study.[10]

## Materials and Methods

Paracetamol BP (Zulat Pharmacy, China), gelatin (R&M Chemicals), maize (National starch & chemical). Potassium dihydrogen orthophosphate ($KH_2PO_4$, Fisher Scientific, UK) and disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$, Merck, Germany) were used. Sodium hydroxide (NaOH, A.R grade, purity 99%, MW 40, batch no. 020606 JRL, System®) was used to prepare 0.1M sodium hydroxide solution. All other materials and reagents were of analytical grade.

### Preparation of Gelatin-Paracetamol Matrices

Microwave power from 10-100% and irradiation time from 10-60s was varied to generate temperature of 60□C [11]. Maximum amount of paracetamol to be added was determined based on its weight (grams) dissolved in predefined volume of water with or without use of sonicator. Time for dissolution of drug was also noted.

Using D-optimal design [12] implanted in DX® Ver 12, amount of maize (as fructose) was changed within 0.5-2.5g, sodium bicarbonate within 2-4g and time of irradiation from 20-50s to generate

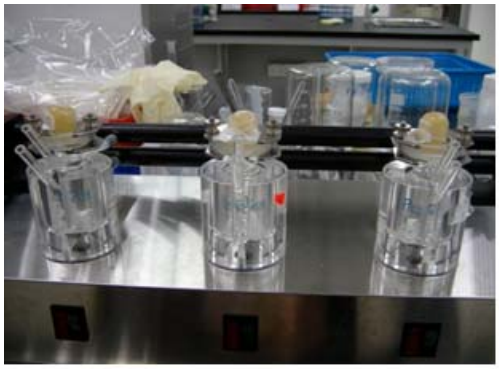
experimental matrix (Table 1). Amounts of paracetamol (0.4g), and gelatin (9.3g), microwave irradiation power (30%) and stirring time (30s) were fixed. All above ingredients were dissolved with stirring for 30s in 22.2-26.2 ml of water to keep total number of parts as 38.4. The resultant mass was exposed to 30% microwave power (Sharp, input-1.60 kW, output- 1100W, frequency-2450MHz and equipped with magnetron emitter) for 50s to achieve 60°C, to sustain stability of drug. Twenty matrices were prepared and incubated (Memmert, type BE 500, 230V, 3.9A, 50/60Hz, 900W, Germany) for 17 h at 37°C.

**Table 1:** Experimental layout for producing optimized paracetamol matrices

| Matrix | Time of irradiation (s) | Maize (g) | Na Bicarbonate (g) | Water (ml) |
|---|---|---|---|---|
| 1 | 20 | 1.5 | 4.0 | 23.2 |
| 2 | 20 | 0.5 | 2.0 | 26.2 |
| 3 | 35 | 0.5 | 3.0 | 25.2 |
| 4 | 50 | 0.5 | 4.0 | 24.2 |
| 5 | 20 | 0.5 | 4.0 | 24.2 |
| 6 | 50 | 2.5 | 4.0 | 22.2 |
| 7 | 50 | 1.5 | 3.0 | 24.2 |
| 8 | 50 | 0.5 | 4.0 | 24.2 |
| 9 | 50 | 0.5 | 2.0 | 26.2 |
| 10 | 50 | 2.5 | 2.0 | 24.2 |
| 11 | 35 | 2.0 | 3.5 | 23.2 |
| 12 | 50 | 0.5 | 2.0 | 26.2 |
| 13 | 20 | 0.5 | 2.0 | 26.2 |
| 14 | 20 | 2.5 | 2.0 | 24.2 |
| 15 | 20 | 2.5 | 3.0 | 23.2 |
| 16 | 35 | 1.5 | 2.0 | 25.2 |
| 17 | 50 | 2.5 | 2.0 | 24.2 |
| 18 | 35 | 0.5 | 2.0 | 26.2 |
| 19 | 50 | 2.5 | 4.0 | 22.2 |
| 20 | 20 | 2.5 | 4.0 | 22.2 |

***In-vitro* drug release study**

*In-vitro* drug release study of paracetamol was performed with Franz diffusion cell (PermeGear, Inc. USA, Fig 1A) fitted with thermostat (CC1, Huber, D77656, Fig. 1a) in phosphate buffer, pH 5.8, set at 37□2□C utilizing Visking membrane (VM), of thickness 0.0145 cm, pore size 0.45µm (Whatman® International Ltd Maidstone, England) was used. Prior to experimentation, VM was treated by heating in 2% sodium bicarbonate and 1mM EDTA at 80□C for 30 min and rinsed with dissolution media.[13, 14] Receiver compartment was loaded with dissolution medium and magnetic stirrer, rotated at 300 rpm. Membrane was mounted between donor and receptor compartments and was clamped (Fig. 1b). The conditions for release study (pH, membrane type, membrane treatment and rpm of magnetic stirrer) were set for maximum release after screening experimentations.



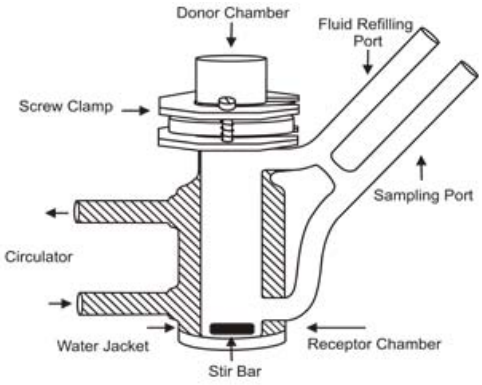



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**Figure 1:** Franz diffusion cells

Donor compartment was filled with sample and covered with parafilm to prevent sample leakage. Sample of 1 ml was withdrawn at 5, 15, 30, 45 min and then at 1, 2, 3, 5, and 8h from receiver compartment and replenished with fresh dissolution media [15]. Paracetamol concentration was determined with UV spectrophotometer (Shimadzu, model 1240, Japan) at 244nm using calibration curves in respective media[10].

**Preparation of optimized and control gelatin-paracetamol matrices**

Percentage drug released, CV and MDT were calculated and release mechanism was noted by PCP Disso v3. All outputs were entered in generated template and analyzed using DX®. Best mathematical model for each response was selected based on statistical parameters. Best formulation based on best levels of factors given by DoE was prepared and labelled as validation formulation and drug release was studied [16]. Control gelatin matrices were prepared with optimized factor levels, but instead of microwave heating, exposed to classical heat by hot plate equipped with digital magnetic stirrer (MSH-20D, Daihan Labtech Co. Ltd., WiseStir®). Target temperature to be achieved was 58°C.[10]

**Thermal Analysis of control and optimized matrix**

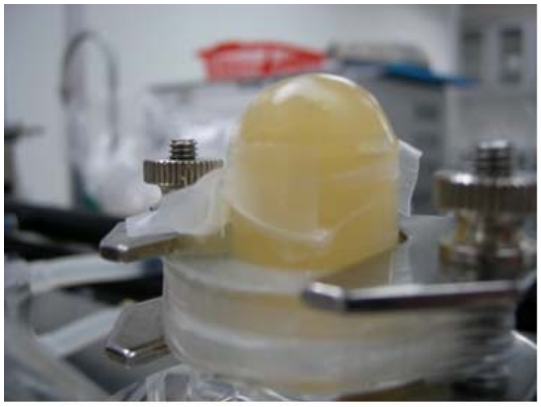
Differential scanning calorimetry (DSC, Mettle Toledo, Switzerland) was used to perform thermal analysis of control and optimized matrix. Sample weights 5mg -10mg were crimpled in standard aluminum pan by using crucible sealing press, hermetically sealed aluminum pans were heated from 20°C-200°C rate of 10°C/min under constant nitrogen flow at 20 ml/min to record DSC thermograms.

**Statistical Analysis**

Release data after classical heating (control) and microwave heating was compared using Mann-Whitney test statistics with statistical software (SPSS) at significance level of p-value $< 0.05$.

**Results**

Paracetamol of 0.4g was dissolved in 24 ml water with use of bath sonicator for 45 min and for bringing about cross-linking, microwave power and radiation exposure was varied resulted into smooth and swellable paracetamol gel matrix (Fig. 2a). A minimum of 30% power of microwave on exposure to microwave energy for 50s, helped attaining 60°C.



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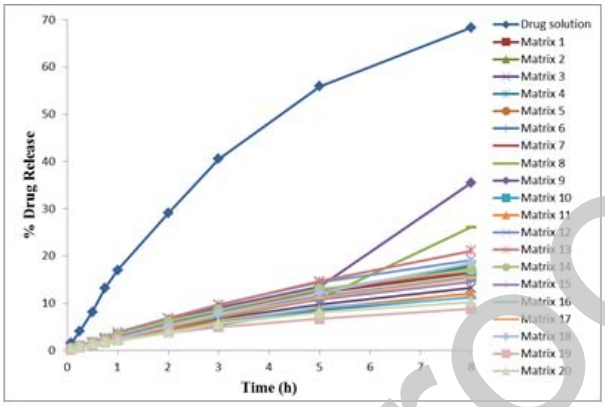


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**Figure 2: (a)** Paracetamol swellable gel matrices **(b)** % Drug release of paracetamol solution and cross-linked matrices 1 to 20 designed by DX® over 8h.

Fig. 2b shows pure paracetamol solution achieved 68.35% drug release (permeation) until end of 8h compared 8.82% -35.51% from all cross-linked matrices. Cross-linked matrices 8 and 9 showed higher drug release after 6h. CV in drug release was comparatively higher for majority of matrices at 0.083 h which was reduced at later time intervals. MDT increases from 0.04 h-5.05h after 8h. Based on the maximum value of coefficient of relationship ($R^2$) values paracetamol release from microwave-treated cross-linked matrices followed Peppas model except matrix 18, which was fitted to first order kinetics. A slight decrease in %drug release at 1h appeared when irradiation time was increased from 20-35s at low levels of cross-linker (Fig. 3a to 3e). Mean dissolution time (MDT) at 1, 3 and 8h (Fig. 4a to 4d) were lower in matrices having high concentrations of salt and cross-linker with microwave exposure of 50s. Response plot showed saddle shaped surface for MDT at 1 and 5h. Same decreasing trend of MDT was seen till 8h. CV increased from 3.51 to 14.83 accompanying with increase in duration of microwave from 20s to 50s at 3h (Fig. 5a to 5d). Increased levels of maize did not affect CV. Moderately high levels of sodium bicarbonate with accompanying higher microwave exposure led to increase in CV.

DoE-predicted combinations of maize, sodium bicarbonate and radiation exposure time for desired properties of outputs such as lower release, least CV and lower variations in drug release were 2.50g, 2g and 45s, respectively. The amount of gelatin, drug, and water were fixed as 9.30g, 0,40g and 24.2 ml. Based on the composition and condition, optimized and control matrices showed responses as given in Table 2.

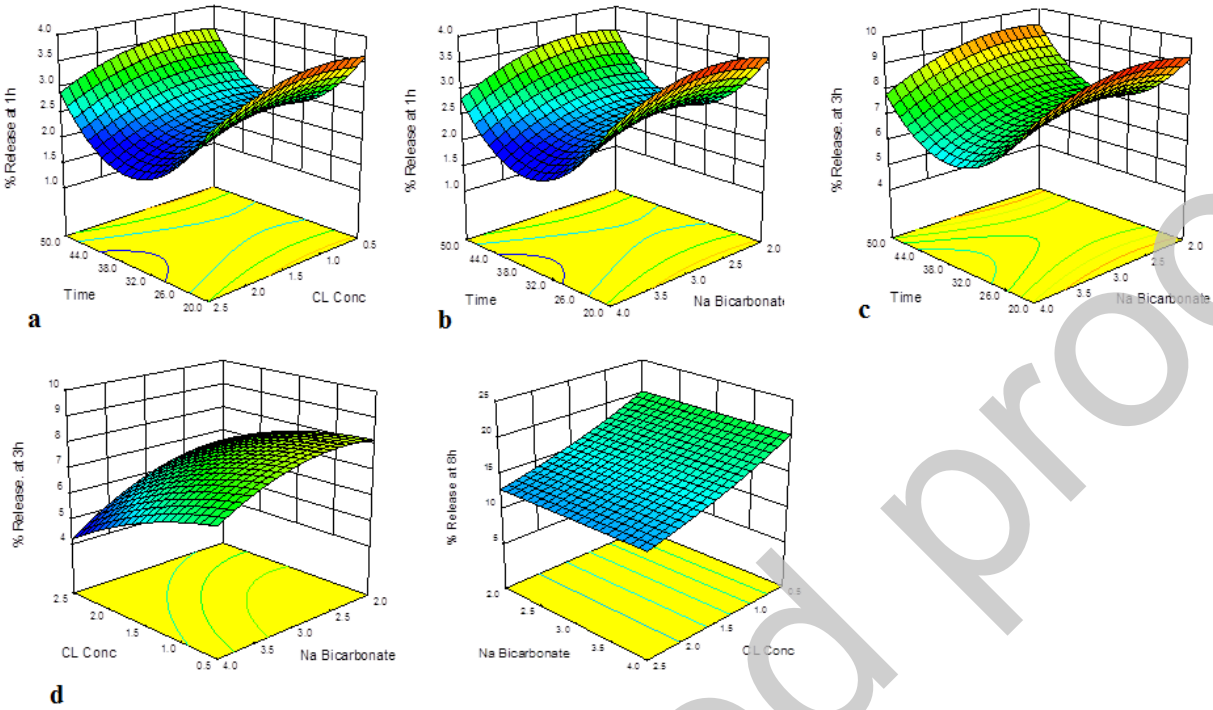



**Figure 3:** Combined effect of (**a**) Irradiation time and cross-linker concentration (**b**) Irradiation time and sodium bicarbonate at 1h (**c**) Salt and irradiation time (**d**) Salt and cross-linker on drug release at 3h (**e**) Cross-linker and salt at 8h

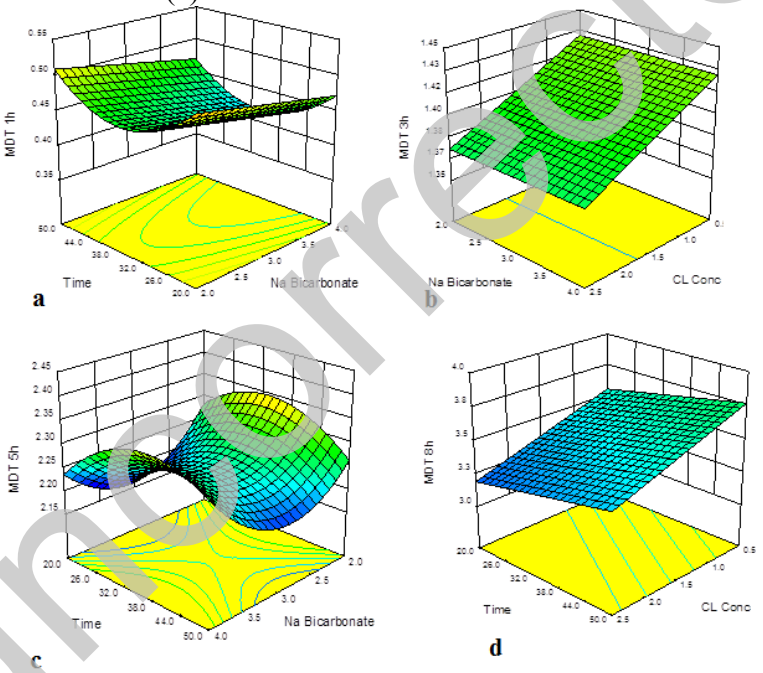



**Figure 4:** Combined effect of (**a**) Irradiation time and cross-linker concentration on MDT at 1h (**b**) Irradiation time and sodium bicarbonate at 3h (**c**) Salt and irradiation time at 5h (**d**) Irradiation time and cross-linker on MDT at 8h

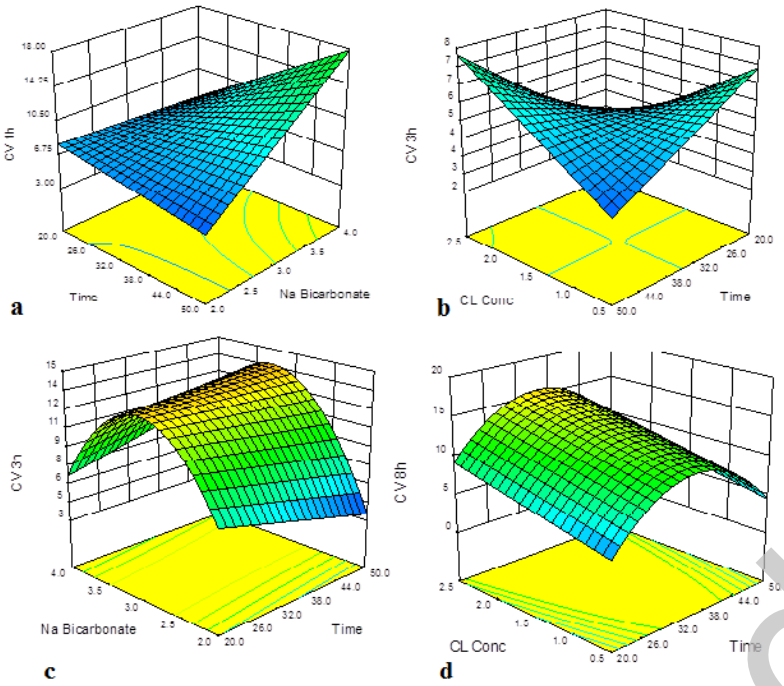
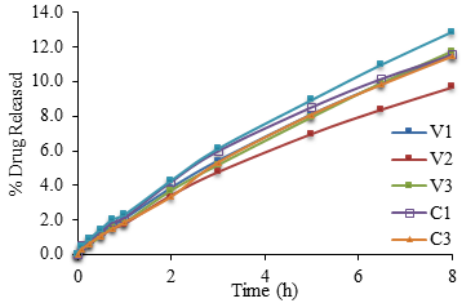



**Figure 5:** Combined effect of (**a**) Irradiation time and sodium bicarbonate on (CV) at 1h (**b**) Cross-linker concentration and time (**c**) Salt and irradiation time at 3h (**d**) Cross-linker and time on CV at 8h

**Table 2:** Responses of optimized and control formulations

| Time (h) | Mean SD | | | | | |
|---|---|---|---|---|---|---|
| | Release (%) | | Coefficient of variance (CV) | | Mean dissolution time (MDT) | |
| | Validation formulations | Control formulations | Validation formulations | Control formulations | Validation formulations | Control formulations |
| 0.083 | 0.259 0.0566 | 0.403 0.1159 | 34.325 28.9560 | 27.993 21.8610 | 0.040 0.0000 | 0.040 0.0000 |
| 0.25 | 0.597 0.0715 | 0.780 0.1562 | 22.813 15.9570 | 17.630 16.9270 | 0.113 0.0058 | 0.103 0.0115 |
| 0.5 | 1.108 0.0953 | 1.287 0.1888 | 17.240 9.4518 | 20.037 9.1173 | 0.237 0.0115 | 0.210 0.0200 |
| 0.75 | 1.577 0.0904 | 1.770 0.2816 | 13.897 6.3350 | 17.317 9.5348 | 0.350 0.0173 | 0.323 0.0115 |
| 1 | 1.866 0.1543 | 2.073 0.2272 | 12.830 6.2367 | 16.820 10.5052 | 0.430 0.0100 | 0.403 0.0321 |
| 2 | 3.639 0.2180 | 3.923 0.5237 | 8.360 2.6254 | 17.857 11.9501 | 0.953 0.0252 | 0.923 0.0153 |
| 3 | 5.102 0.3239 | 5.793 0.4143 | 6.730 1.2869 | 17.673 10.7575 | 1.397 0.0115 | 1.433 0.0666 |
| 5 | 7.658 | 8.513 | 7.865 | 18.960 | 2.267 | 2.257 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 0.6042 | 0.4153 | 2.6375 | 7.9269 | 0.0503 | 0.1002 |
| 8 | 10.993 ± 1.1533 | 11.957± 0.7836 | 7.233 ± 0.9347 | 18.350 ± 6.1724 | 3.533 ± 0.1350 | 3.460 ± 0.1493 |

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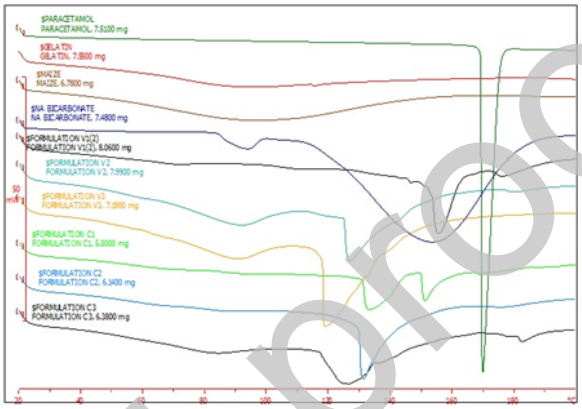

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**Figure 6: (a)** %Drug release of validation and control formulations (b) Combination of ingredients, optimized and control formulation thermograms

Three control matrices were prepared using the same optimized factor levels, but by classical heating at 60□C, instead of microwave heat exposure. Drug release of optimized and control formulations have been compared in Fig 6a showing almost similar %drug released and best described by Peppas model. For majority of release time points, Mann-Whitney test revealed no difference between classical heating (control) and microwave heating as shown by $p>0.05$. Only % release at 5h, CV at 5h, 8h, and MDT at 0.75h show significant difference between optimized and control formulations ($p<0.05$). Matrices exposed to classical heat demonstrated higher standard deviation as compared to microwave treated matrices. Fig. 6b shows interaction of formulation ingredients and drug in optimized and control formulation. Peak temperature of paracetamol was shifted from 169.79□C to 120□C-160□C as other components were added in formulation. Generally, optimized formulations had two major peaks, which were located in ranges of 69.65□C-91.89□C and 118.81□C-155.63□C. Control formulation had one major peak at range of 126.57□C-133.28□C. Combination thermogram for all optimized samples showed some differences in terms of peak temperature.

**Discussion**

Temperature of 60□C was the maximum effective temperature capable to bring about cross-linking was attained with minimum level of microwave energy (30%) in shorter time duration (50s). All matrices, except 8 and 9 showed up to 35.51% paracetamol release at 8h while release of paracetamol from matrices 8 and 9 was higher after 6h, might be ascribed to variations in drug diffusivity behavior as well as possible structural damage of gel matrices during dissolution.[17] Use of cross-linker and microwave energy reduced release which seemed to be optimal extended release pattern from resulting cross-linked swellable gelatin matrices. Higher CV in drug release for majority of matrices at initial time interval was reduced at terminal time intervals, might be due to reduction in porosity leading to compact cross-linking in matrices. Increase in MDT after 8h suggested that dissolution of paracetamol from gel matrices was considerably slower

compared to pure paracetamol solution. Drug release from all microwave-treated cross-linked matrices was best described by Peppas model except Matrix 18, which followed first order kinetics. Diffusional exponent n between 0.5-1 supported combined involvement of Fickian diffusion and macromolecule chain relaxation (anomalous or non-Fickian transport) during drug dissolution [18]. Due to space constraints, only response surface plots exhibiting prominent effects on drug release are shown. Drug release was considerably controlled by microwave exposure, concentration of maize and sodium bicarbonate which was actually caused by cross-linking of gelatin on providing suitable combination of factors consistent with earlier report [10] stating that cross-linked gelatin product required presence of suitable combinations of all components (gelatin, sugar, and salt) to effectuate cross-linking. The drug release was decreased at 1h on increasing irradiation time at low level of cross-linker, at higher levels of salt and cross-linker 1 and 3h and when both, salt and cross-linker levels were higher with shorter exposure time. High irradiation time alone failed to reduce drug release at 8h when lesser amount of cross-linker and salt were present in Matrices. Our findings were in accordance with the previous reports [6] in which brief duration of microwave irradiation imparted sustained release characteristics to ketoprofen matrix beads. Contrarily, longer irradiation conferred immediate release features, possibly due to effects of irradiation time on matrix porosity as well as on solid state of drug in gel matrix. MDT at 1, 3 and 8h were lesser at higher level salt, cross-linker and microwave exposure. A saddle shaped response plot of MDT at 1 and 5h, showed a non-linear effect of above factors on MDT. Duration of microwave exposure increased CV while moderately high levels of sodium bicarbonate could be effective in increasing CV only at higher microwave exposure. Thus, low CV was related to comparatively shorter regime of microwave irradiations suggesting high precision and reliability under microwave heating conditions. Controlling and limiting variations in outputs shows consistent quality of products and is critically required in research and industrial settings.[19, 20] Low % drug release, low CV, and high MDT were the desired characteristics for optimized matrices. A higher value of MDT shows good release retarding ability of cross-linked system.[21] Three control matrices were prepared by classical heating at 60°C. Controlled and optimized formulations had almost similar %drug released and followed Peppas model. Heating, either classical or microwave-assisted, has facilitated cross-linking among macromolecular (gelatin) chains in presence of cross-linker and salt. The decrease in %drug release from gelatin matrices prepared using 20-60s exposure to 330W microwave energy was an indirect evidence of cross-linking. However, microwave exposure in this study was briefer contrary to longer exposure time of 10 min previously reported [4] that generated temperature of 150–250°C in preparation of gelatin microspheres. Gelatin is sensitive to and is maneuvered at lower temperatures [22], particularly in presence of cross-linker also observed in present study. Appropriate concentration of cross-linkers (genipin and glutaraldehyde) improves mechanical strength and thermal stability of gelatin films [23]. In this study, cross-linking was resulted from chemical interaction between sugar (maize) and gelatin under controlled heating. According to Cortesi *et al*. [24] sugars react with gelatin via two main reactions. One reaction is called Amadori rearrangement, in which gelatin amino group (lysyl ε-amino group) reacts with aldehyde group of sugar to produce a cationic imine that rearranges to form a methylene link between 2 ε-amino groups of lysine, hence forms a cross-linked structure. In other reaction, carbonyl group of sugar open-chain form reacts with free amino groups of gelatin molecule, after a number of tautomerizations, in formation of a ketose sugar. This carbonyl adduct can further associate with another amine group forming a cross-linked structure [24]. The DSC thermograms supported the crosslinking (Figure 6B). The pure paracetamol showed a sharp melting endotherm

at 169.79 °C due to crystalline nature of drug, consistent with literature [25]. DSC curves of maize and gelatin showed a shallow endotherms due to their poor thermal conductivity [26] illustrating a gradual structure loss over time. The DSC curves of microwave heat treated (V1, V2 and V3) and classical heat treated formulations (C1, C2 and C3) exhibited curve with variable endothermic peak positions. Overall peak broadening with decreased melting temperatures in the range of 126.57 °C-133.28 °C was observed, probable due a decreased drug crystallinity led by drug-polymer crosslinking. The findings also probably demonstrated different levels of cross linking with different types of heat treatments to the samples as depicted by varied peak positions. Control of the method parameters such as heating rate, placement of sample, amount of sample either in classical or microwave heating methods probably influenced the thermal behavior of the matrices. As expected relatively better results in terms of standard deviation, i.e., lesser varied peak positions were observed in case of microwave heating method.

No difference between matrices prepared using classical heating (control) and microwave was found. Optimized and control matrices were different with respect to couple of parameters, such as %release at 5h, CV at 5h, CV at 8h, and MDT at 0.75h. Interestingly, output of microwave exposure was having low variations (indicated by low standard deviation) and of consistent quality achieved within shorter time. Consistent quality for a sustained release carrier could ensure consistent drug delivery in terms of correct timing and accurate dosage amount. This discriminative effect of microwave irradiation was probably due to its ability to penetrate heat uniformly, thereby facilitating an appropriate aldehyde cross-linking with amino group of gelatin.[4] Thermal analysis using differential scanning calorimetry indicated interaction between paracetamol and other ingredients as shown by left peak shift. A previous study reported the chemical stability of drug embedded in matrix generated by microwave irradiation. Hence, paracetamol in this study was expected to sustain its stability[27, 28] Though combined thermogram for all optimized samples showed some differences in terms of peak temperature, however, endothermic enthalpy may be caused by duration of storage of samples.[29] The microwave may be an effective approach in future prospective.

**Study Limitations**

The time for the complete release paracetamol was not determined. The non-thermal effects of microwave irradiation must also be assessed.

**Conclusion**

Cross-linked gelatin treated with microwave was able to sustain release of paracetamol as compared to paracetamol solution alone. As indicated by relative standard deviation in formulations, microwave treated cross-linked gelatin formulations gave consistent release and lower variations in release data as compared to that prepared by classical heating. DoE could be used as a tool to optimize conditions. Finally, it can be suggested that microwave heating can be applied to produce consistent quality of pharmaceutical products.

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