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DELIVERY OF WATER-INSOLUBLE DRUGS: CAFFEINE MONOHYDRATE AND SALICYLIC ACID AS MICROCAPSULES USING IRVINGIA GUM AS THE COACERVATING AGENT

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ABSTRACT

The potential of irvingia gum as a coacervating agent was investigated in the microencapsulation of caffeine and salicylic acid. Irvingia gum was produced according to the methods described by previous workers. Coacervation was achieved by adding ethanolic solution of the drug (6g in 8ml) to the gum mucilage (3g in 100ml of water): i.e, gum: drug ratio 1:2, and allowing to stand for 48h to form a sediment (coacervate). Also prepared were gum: drug ratios: 1:1 and 2:1. Effects of composition, temperature and pH on coacervate yield and degree of drug entrapment were investigated. Tragacanth gum was used, as reference standard. 1:2 ratio gave the highest yield for both caffeine (78.5±0.02% w/w) and salicylic acid (79±0.00%w/w) coacervates. pH increase of gum mucilage (2.21 to 12.03) increased the yield of irvingia caffeine coacervate but decreased that of irvingia: salicylate coacervates. Raising the temperature (25-60^{0}C) decreased both coacervate yields. Both systems (with gum: drug ratio 1:2) gave the highest degree of drug entrapment. This study, therefore, presents optimal condition for enhancement of yield and degree of drug entrapment in coacervation of poorly-water soluble drugs: caffeine and salicylic acid.

KEY WORDS: Irvingia gum, coacervation, microencapsulation, water-insoluble drugs.

INTRODUCTION

Coacervation is a desolvation process resulting in the solid phase separation, achievable by addition of a non- solvent for the solute but which is miscible with the solvent [1,2 ,3 4, 5]. For instance, addition of excess water to an ethanolic solution of a water-insoluble drug will result in precipitation of the drug from solution (i.e. separation of the solid phase from the liquid phase). Where a water soluble polymer and a test drug are coprecipitated, the coacervation process can be applied for the miroencapsulation of the drug particles as cores in a polymeric coat for retard release application [6,7] . On the other hand, a highly water soluble substance such as the hydrocolloid gums can be coprecipitated with a water insoluble drug by addition of an organic solution of the drug to the aqueous hydrocolloid solution. Such coacervates dissolve rapidly [8] hence coacervation is also a technique for the enhancement of the dissolution rates of water insoluble drugs. In this age of technological advancement especially in drug delivery system, the researchers deemed it necessary to work on irvingia gum as a coacervating agent in the microencapsulation of water insoluble drugs: caffeine monohydrate and salicylic acid- a study aimed at contributing to drug delivery. Recent studies have been made on irvingia gum namely: "Preparation and physical characterization of irvingia gum powder obtained from the seeds of *Irvingia gabonensis*" [9] "Screening of irvingia gum powder for elemental and carbohydrate contents" **[10]** "Moisture uptake potentials of irvingia gum powder and the effect on its particle structure" [11] and

"Investigation of the binder property of irvingia gum(novel) in tableting and the effect on tablet dissolution rates." [12]

Irvingia gum powder hydrates slowly in water to a viscous mucilage but the mucilage is readily flocculated by addition of organic solvents such as ethanol and propanol or by adjustments of the pH of the mucilage to the iso- electric point (pH 5.85). Thus irvingia gum has a potential as a coacervating agent and can be coprecipitated with water insoluble but ethanol soluble drugs such as caffeine monohydrate (a weakly basic drug) and salicylic acid (a weakly acidic drug). The interaction of the gum with the drug to form the coacervate particle will be determined by the degree of ionization of the drugs.

Consequently, the influence of pH of the coacervating medium on the yield of coacervates was an important consideration in this study. Other factors considered to affect the yield and degree of drug entrapment were: the gum to drug ratio and temperature of coacervation. The system consisted of water as solvent for the gum but a non-solvent for the drug (salicylic acid or caffeine) and ethanol was the solvent for the drugs but was the non solvent for the gum.

MATERIALS AND METHODS

Materials: Irvingia is a fine to moderately coarse powder, tasteless with a characteristic odour, and has a light brown colour. It is obtained locally by defatting milled seeds of *Irvingia gabonensis.* Details of the extraction procedure have been described elsewhere [13]. It is slowly hydrated in aqueous medium to form a viscous liquid. Caffeine monohydrate and salicylic acid (both from BDH, Poole, England and of BP grade) were the test drugs, ethanol 95%w/v was the solvent for the drugs; tragacanth gum powder (BDH, Syria) was included in the study as reference standard.

METHODS

Preparation of irvingia gum: The testa of the seeds were first removed and the seeds milled to a coarse powder using a hand driven milling machine (03200 Landers &Ciasa,Corona). A sample of the milled seeds (220g) was macerated in 1500ml of a solvent mixture consisting of chloroform and methanol in the ratio of 2:1 for up to 10h, stirring occasionally with a spatula. This solvent system and the maceration time, 10h, were selected for effective defatting of the milled seeds based on the procedure by [13]. The supernatant solvent mixture was decanted and the residue (i.e. the crude gum) was strained to remove excess solvent. The crude gum was soaked in hot water overnight to allow complete hydration to a colloid. The colloid was strained through a filter cloth, and concentrated to 10mg/ml by evaporation (using water bath mantained at 60^0C) for 12h. The gum was extracted by treating the colloid with thrice its volume of acetone (i.e. ratio 1:3) in aliquots. The extracted (purified) gum was dried to moisture content of 3 ± 0.58.%w/w in a hot air oven at 60^0C for 1h. The yield of the pure gum powder was 25±2.3%w/w of the milled seeds. The gum was stored in an airtight container 24 h before use. The experiment was done in triplicate and mean yields of gum computed.

Test for complete removal of fat from irvingia gum

A sample of the purified gum (10g) was added to 227ml of the acetone/benzene/water mixture (ratio 97:30:8) [14], and stirred for 10mins with a glass rod. The mixture was filtered and the filtrate was subjected to TLC analysis for presence of fat using wool fat as standard [14]. The test was repeated with milled seeds and from the difference the degree of defatting (%) was calculated. Extent of defatting of irvingia gum was complete as its Rf value was zero, while those of wool fat and milled irvingia seeds 0.563 and 0.457 respectively.

Identification tests: 1). 1g of irvingia gum powder was dispersed in 100ml of cold water and the effect observed. [14,15]. A viscous dispersion was obtained which suggests the presence of gum.

(2.) 5ml of 10% dispersion of irvingia was added to 1ml of dilute lead subacetate solution and the effect noted [14]. A white precipitate confirmed the presence of gum.

Coacervation Technique

1) Preparation of coarcervates: The methods developed by [16,17 18] was employed but with slight modification. The gum mucilages of varying concentrations (2% w/v to 6%w/v) of irvingia and tragacanth gums were prepared by dispersing a sample of the gum powder in water (100ml) and allowed to stand for 48h for complete hydration and gelling. The mucilage was then homogenized stirring with a Silverson mixer (model A X R, England) at 100 rpm for 15min. Ethanolic solutions (750mg/ml) of the test drugs (caffeine and salicylic acid) were made. To coacervate, a sample of the ethanolic solution, (containing various concentrations of either caffeine or salicylic acid), was added to 100ml of the gum mucilage with continuous stirring for 5min, during which precipitates (gum-drug coacervates) were formed and were collected by filtration after standing for 24h, washed with ethanol (3ml) and air-

dried at room temperature (30°C) for 10h. The dried coacervate particles were stored in airtight containers in a desiccator for 24h before their evaluation The experiment was carried out in triplicate and mean yield of coacervates expressed.

2). Factors which affected coacervate yield: The variables that may affect yield which were investigated included: composition (gum: drug ratio), pH, and temperature.

(a) Composition (gum: drug ratio): The process of coacervation was carried out using the following gum: drug ratios 1:2, 1:1, and 2:1. Following coacervation, the mixture was stirred vigorously, and a sample (10ml) was immediately transferred to a 10ml-measuring cylinder and allowed to stand (2h) until there was no change in volume of the supernatant and the sediment (coacervate layer). The equilibrium coacervate layer (i.e. volume) was noted and the degree of coacervation (% yield) was expressed as the % v/v of the coacervate system. The experiment was done in triplicate and the mean results used to draw a graph of % coacervate yield versus composition. Each coacervate system was examined under scanning electron microscope and photographs taken of representative fields of view.

b) pH of the coacervation medium: The pH of the gum mucilages were varied by the addition of acetate buffer and phosphate buffer tablets to 250ml of distilled water to obtain aqueous solutions ranging from acidity (2.21) to alkalinity (12.03). Varying weights of the gum powder was dissolved in 100ml of each solution and the process of coacervation repeated in triplicate. Graphs of % coacervate yield versus pH were plotted.

c) Temperature of coacervate medium: The temperature of the fluids (i.e. the gum mucilages) was varied (28°C to 60°C) by storage in a refrigerator until the temperature fell from 30°C (room temperature) to 28°C or by storage in a water bath 2h before coacervation The experiment was carried out in triplicate. Graphs of % coacervate yield versus temperature were constructed. .

Assessment of degree of drug entrapment: The amount of drug sequestered from the organic solution into the coacervates, was determined by assaying the supernatant for content of free drug, using spectrophotometric methods described by [19]. The entraped drug was obtained by difference from the initial amount of drug employed in the coacervation. The degree of entrapment was expressed as the %w/w of the initial amount of drug used in the coacervation. Initially, a standard calibration curve was each prepared for the test drugs: caffeine monohydrate and salicylic acid (at λ max 272nm and 540nm respectively). 1%w/v ferric chloride solution was used to colour the salicylic acid solution. From the curve, the amount of drug present in the supernatant was obtained.

Determination of drug content in the dried coacervates: A standard calibration curve of caffeine and salicylic acid was each prepared as follows: 100mg of caffeine or salicylic acid was dissolved in 100ml of 0.1N HCl (the dissolution medium). Serial dilutions of the stock solution were made to obtain 1, 2, 3, 4, 5, 6, 7, 8 and 9µg/ ml. The absorbance of the standard solutions were determined at λ max 272nm and 540nm for caffeine and salicylic acid respectively using the UV spectrophotometer (Model Spectronic 21D, Bausch and Lomb,U.S.A.).The test was carried out in triplicate and mean absorbances obtained. Plots of the mean absorbances versus concentrations were made. A sample of the dried coacervates (100mg) was crushed in a mortar and triturated with about 6ml of 0.1N HCl. The triturate was transferred with rinsing to 100ml measuring cylinder and made up to volume with 0.1N HCl. After stirring for 5 min, the mixture was filtered using No.1 whatman filter paper. The filtrate was assayed spectrophotometrically at λ max 540nm (salicylic acid) and at λ max272 for caffeine. The experiment was carried out in triplicate and the mean results presented. The amount of drug contained in the coacervate was read directly from the standard curve.

Statistical analysis of Datum: The datum was expressed as mean ± SEM. The datum was statistically analyzed by the students' t test. The level of significance was from $p \leq 0.05$.

RESULTS AND DISCUSSION

Factors which affected coacervate yield

Composition (gum: drug ratio): The gum: drug ratios (1:2, 1:1, and 2:1) employed in the coacervation yielded coacervates to varying degrees 78.5%, 43.2% and 32.6% respectively, for irvingia-caffeine coacervates (Fig. 1) as revealed by the scanning electro micrographs of these coacervates (Fig.2). The same was true of irvingia: salicylic acid coacervates (Figs.1 & 2). Tragacanth: caffeine or salicylic acid coacervates followed similar pattern (Fig.1). Generally coacervates of caffeine and salicylic acid (ratio 1:2) gave the highest yield of coacervates, attributable to presence of active sites in 1:2 ratio but which were saturated in the other ratios (1:1 and 2:1) [20]. Also according to [21] better wetting

and hydration of gum particles lead to better association and hence more yield at gum drug ratio 1:2. The yield of caffeine coacervates increased with pH increase (Fig 3.) attributable to flocculation of caffeine at higher pH values due, probably, to the neutralization of surface charges on caffeine, leading to depression of ionization and solubility of caffeine. However, the yield of salicylic acid coacervates decreased with pH (Fig. 3.) attributable to the ionization of salicylic acid (weak acid) at the higher pH resulting in lower gum-drug interaction. In addition, the zeta potential decreases with increase in pH and vice versa. At high pH values, the zeta potential is reduced, leading to flocculation and precipitation, and hence higher yield of coacervates.
Increase in temperature (28^o to 60^o C) resulted in a decrease in coacervate yield (Fig 4) suggesting that the gum-drug interaction was exothermic. Hence, the gum-drug interaction was suppressed at the higher temperatures [22]. Thus, temperature control in coacervation is of paramount importance. The implication of this study is that coacervation should be carried out under optimal temperature conditions to ensure optimum yield.

Factors which affected degree of drug entrapment
Some of the factors that affect degree of drug entrapment (or encapsulation efficiency) include gum type, gum: drug ratio (composition) and nature of drug.

1. Type of Gum: Irvingia and tragacanth gum coacervates exhibited similar patterns of drug entrapment (Fig 5). This is to be expected as the two gums produce weakly acidic dispersions in water (pH 5.95 and 4.61 respectively for irvingia and tragacanth mucilages).

2. Composition (Gum: drug ratio): Systems with gum: drug ratio 1:2 gave the highest degree of drug entrapment (Fig 5) over the other ratios i.e. 1:1 and 2:1. This is to be expected for coacervates with gum: drug ratio 1:2 had the greatest drug content as earlier discussed..

3. Nature of drug: Caffeine and salicylic acid exhibited similar degree of entrapment (Fig. 5). Caffeine is a weakly basic drug while salicylic acid is weakly acidic. It is really surprising that salicylic acid showed similar degree of entrapment as caffeine (a weakly basic drug) under weakly acidic condition, whereas it should have been entrapped to a greater degree. This similarity probably reflects a partial loss or incomplete coating of salicylic acid during micro encapsulation of the drug particles [18]. This study therefore, presents optimal conditions for enhancement of degree of drug entrapment in coacervation of poorly water soluble drugs-caffeine and salicylic acid.

Drug content in the dried coacervates: Results of the study showed that irvingia gum gave significantly higher drug (caffeine or salicylic acid) content than tragacanth (Table 1) $P \leq 0.05$. Both gums gave higher yield of salicylic acid coacervates than caffeine, (Table1). The difference was significant, $P \leq 0.05$. That irvingia gum gave higher coacervate yield is attributable to its smaller particle size, and thus the particles presented larger surface area for gum – drug interaction which therefore, favoured coacervate formation. In turn, salicylic acid coacervates had higher drug content than caffeine coacervates. This is attributable to the differences in their aqueous solubility. Caffeine monohydrate is soluble in hot water (60^oC), [23] salicylic acid is soluble 1 in 550 parts of water, and therefore, salicylic acid will be more easily precipitated as coacervate than caffeine in the aqueous environment. Also, salicylic acid will readily give up ions in the aqueous environment, faster than the more stable caffeine that possesses covalent and trivalent bonds (> C =0 ; N= C <) .

CONCLUSION

The study has shown that irvingia gum is an effective coacervating agent in the microencapsulation of water insoluble drugs: caffeine monohydrate and salicylic acid dissolved in polar organic solvent such as ethanol. The factors that increased yield of coacervate included composition (gum: drug ratio 1:2 as in this study), low temperature, pH (high for caffeine monohydrate and low for salicylic acid.). The degree of drug entrapment was also affected by composition.

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The Almighty God for the gift of life and health and all resources. To Him be the Glory!

Table 1: Drug content in the dried coacervate of composition gum: drug 1:2

pe of gum in the coacervates	Drug content (%w/v) for the test drugs	
	Salicylic acid	caffeine
Irvingia	91± 5.0	89± 2.0
Tragacanth	68 ± 5	60 ± 5

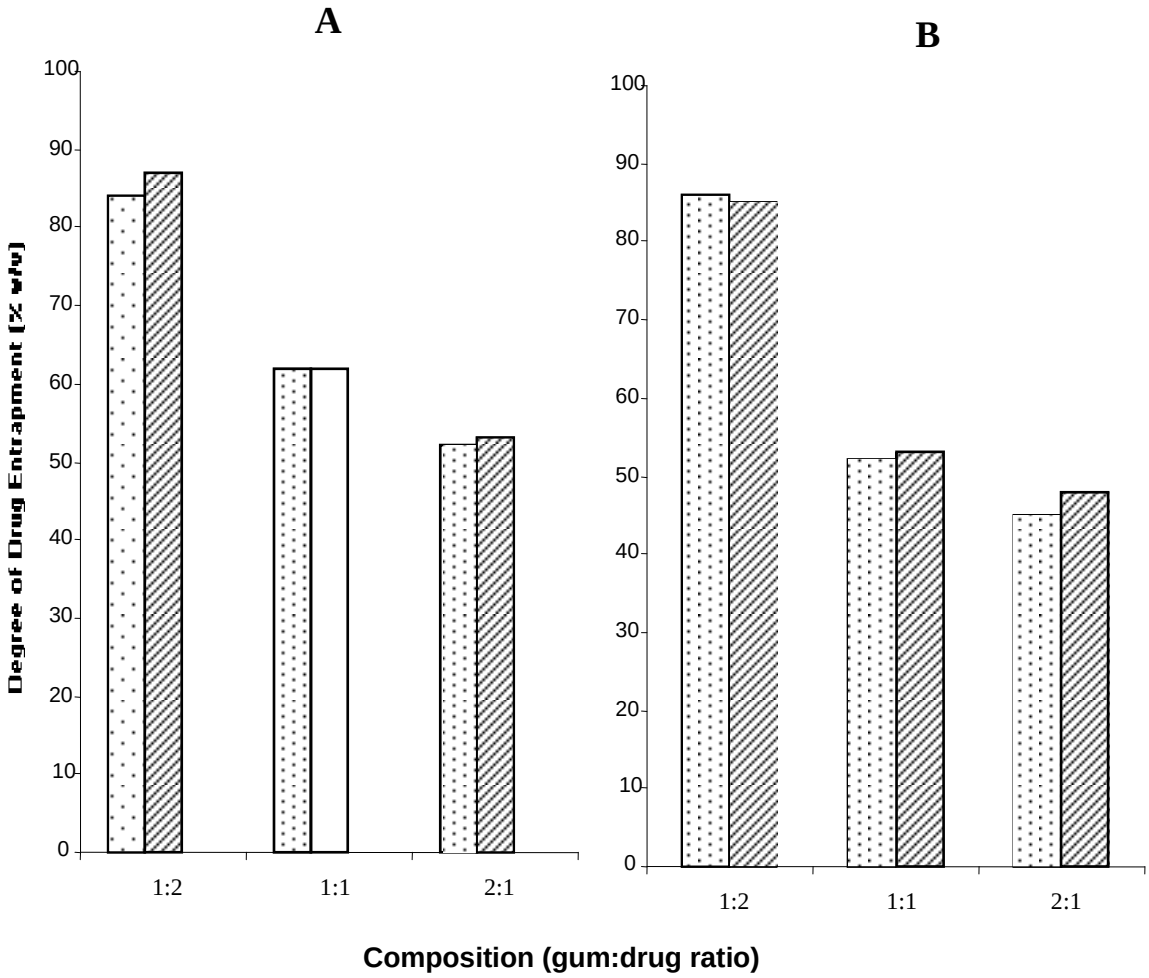
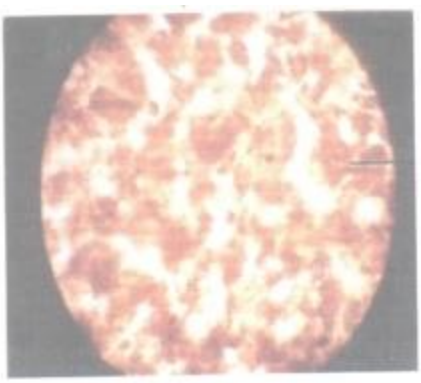
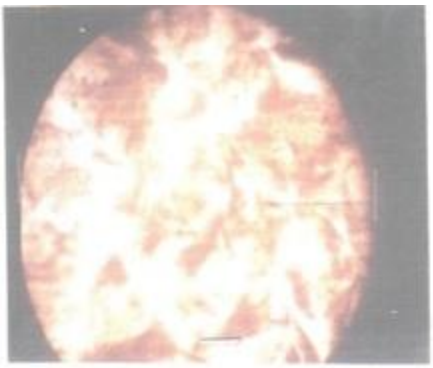


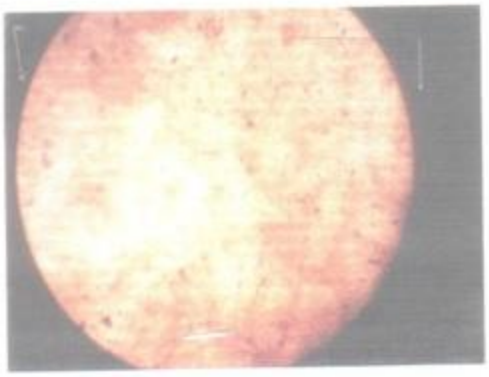
Fig.1: Effect of composition on degree of drug entrapment (%) in the irvingia (A) and tragacanth (B) drug coacervates Test drug: caffeine (⬚) and salicylic acid (▨)



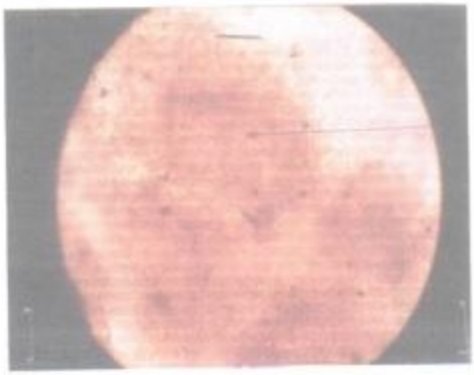
Irvingia: Caffeine (1:2) SEM x 100µ



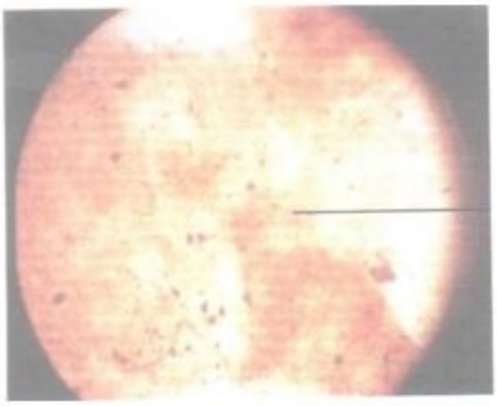
Irvingia: Salicylic acid (1:2) SEM x 100µ



Irvingia: Caffeine (1:1) SEM x 100µ



Irvingia: Salicylic acid (1:1) SEM x 100µ



Irvingia: Caffeine (2:1) SEM x 100µ

Irvingia: Salicylic acid (2:1) SEM x 100µ

Fig.2: Scanning electron micrographs of irvingia coacervate showing effect of composition on coacervation.

One bar represents 1mm.

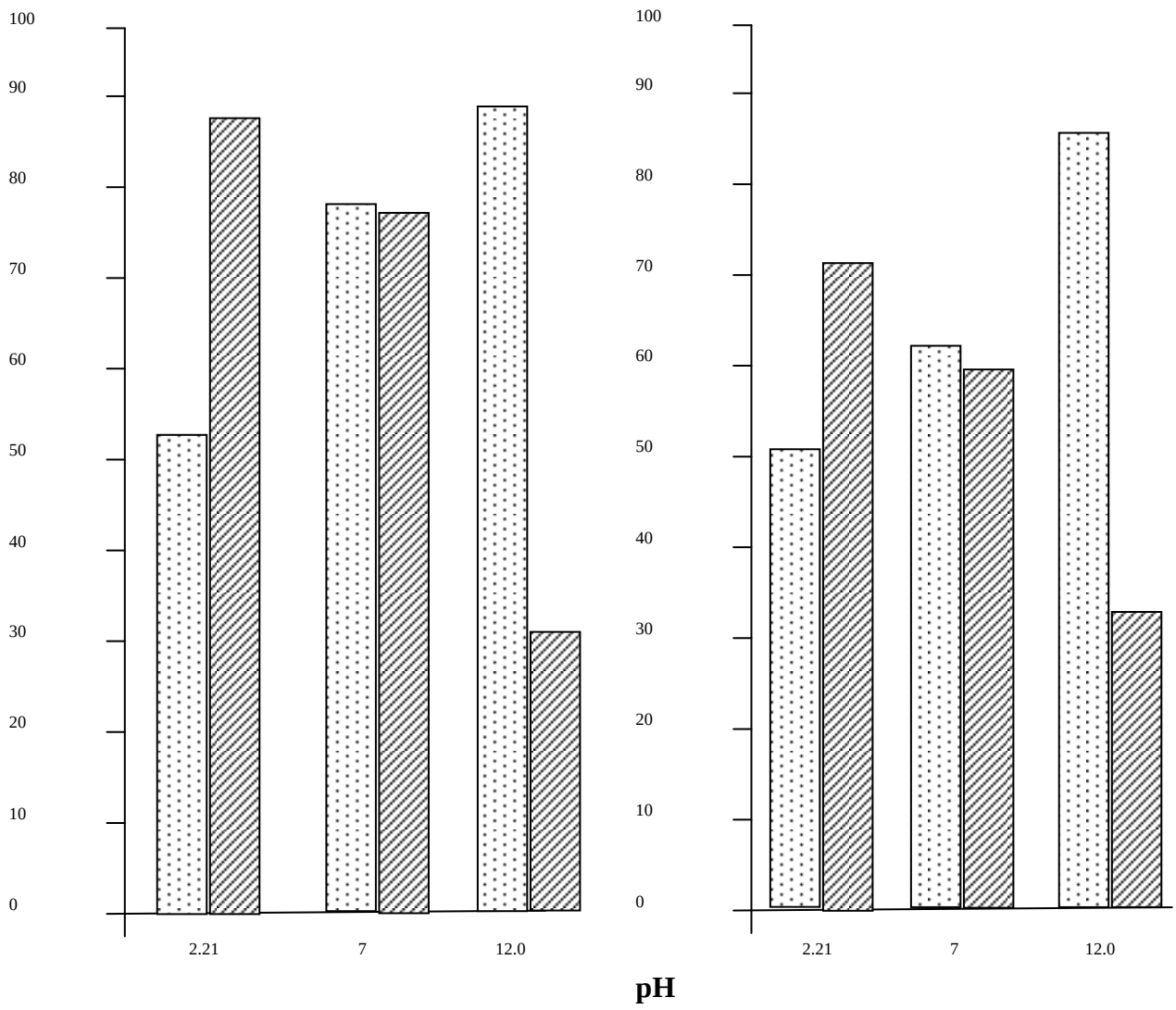


Fig. 3.15: Influence of pH of coacervation medium on the yield %v/v of Irvingia (A) and Tragacanth (B) Coacervates Test Drug: Caffeine ( ) and salicylic acid ( )

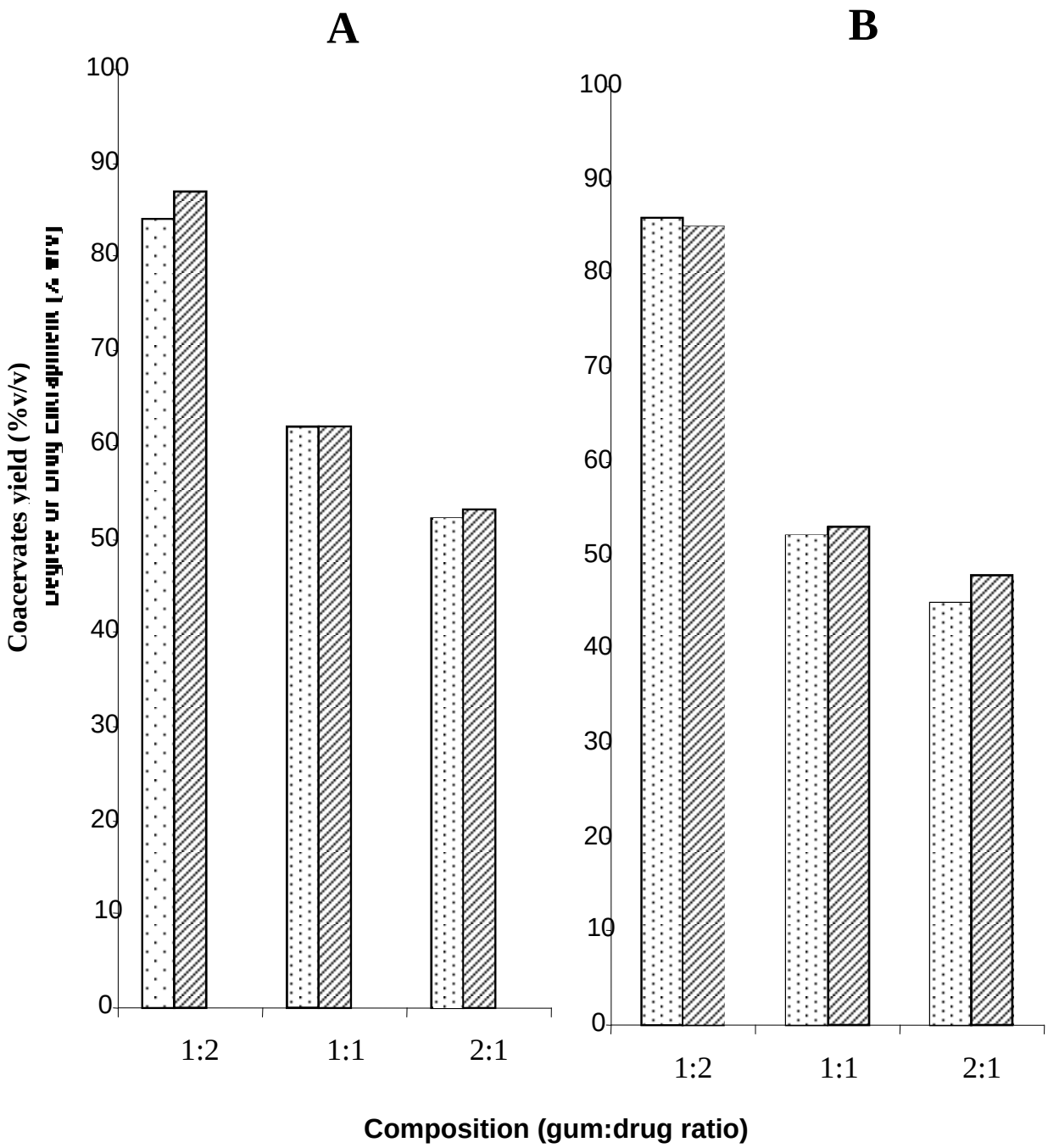


Fig. 3.: Influence of pH of coacervation medium on the yield %v/v of Irvingia (A) and Tragacanth (B) Coacervates Test Drug: Caffeine( ) and salicylic acid ( ) Gum:drug ratio-1:2

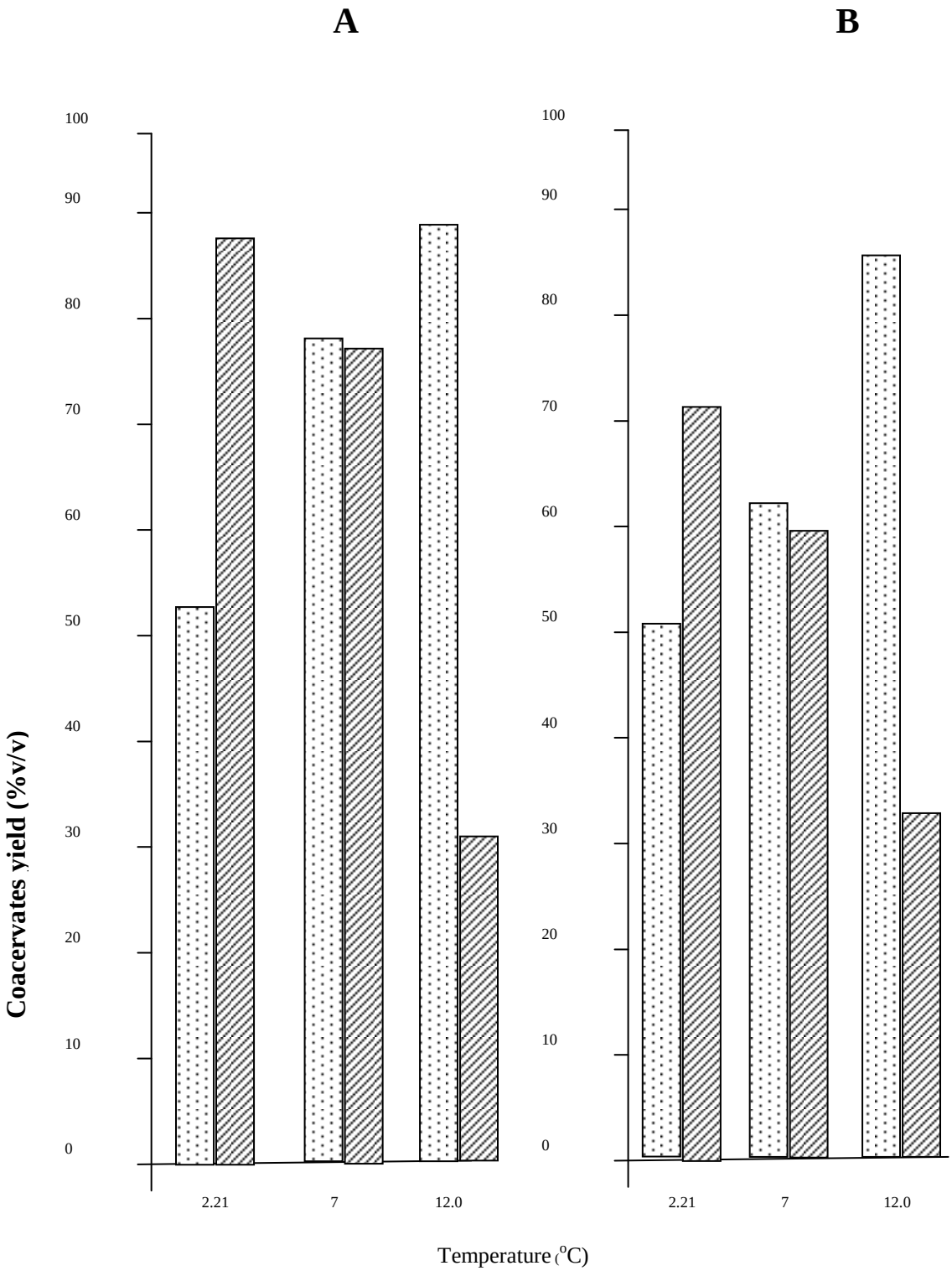


Fig. 4. Effect of temperature of coacervation medium on the degree of yield %v/v of irvingia (A) and tragacanth (B). Test drugs: Caffeine ( ) and Salicylic acid ( ) Gum:drug ratio-1:2

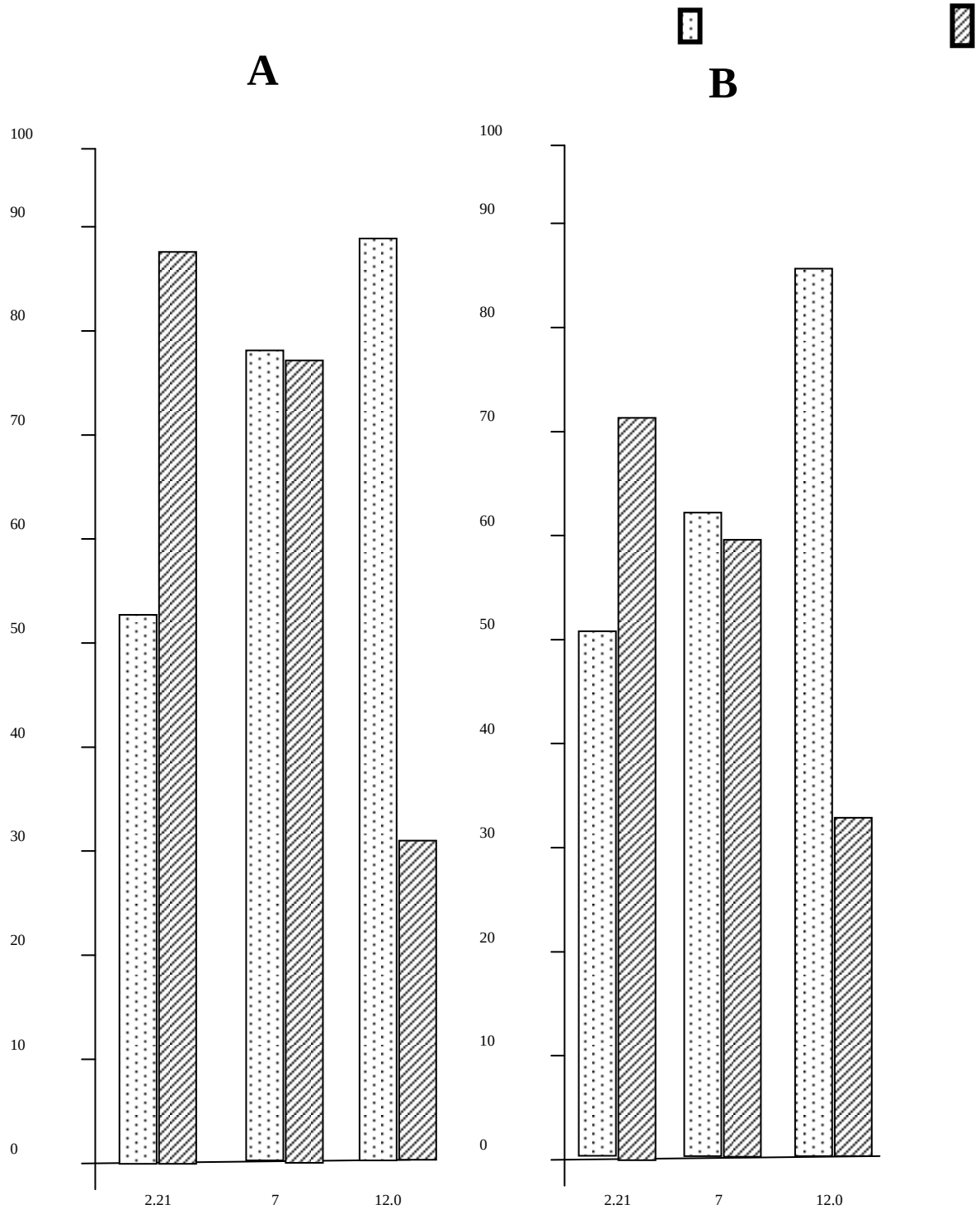


Fig .5 : Effect of composition (gum: drug) on degree of drug entrapment (%) in the irvingia (A) and tragacanth (B) drug coacervates

Test drug: caffein ( ) and salicylic aci ( )

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