Colloid Polym Sci (2004) 282: 1083–1088
DOI 10.1007/s00396-003-1037-4

ORIGINAL CONTRIBUTION

Zhiyong Qian
Sai Li
Yi He
Hailian Zhang
Xiaobo Liu

Preparation of biodegradable polyesteramide microspheres

Received: 25 May 2003
Accepted: 7 November 2003

Z. Qian (✉)
Department of Metal Materials, College of Material Science and Engineering,
Sichuan University, 610065 Chengdu,
China
E-mail: dragonqzy@hotmail.com,
anderson-qian@163.com
Tel.: +86-28-85406380
Fax: +86-28-85460982

S. Li · Y. He · H. Zhang · X. Liu (✉)
Chengdu Institute of Organic Chemistry,
Chinese Academy of Sciences,
610041 Chengdu, China
E-mail: liuxb.cioc@163.com

Abstract Biodegradable polyesteramide copolymer P(CL/AU) based on ϵ-caprolactone and 11-aminoundecanoic acid was synthesized by the melt polycondensation method. Polyesteramide (PEA) microspheres were prepared by a simple O/W emulsion solvent evaporation method. The effects of variations in preparation parameters (such as emulsifier concentration, polymer concentration, polymer solution adding rate, stirring rate, and whether vacuum was applied) were studied in detail. The obtained microsphere morphologies were observed using an optical microscope and via scanning electron microscopy (SEM). The particle size distribution was determined using a Malvern laser particle sizer. When the PEA microspheres were incubated in PBS saline, the particle size increased at first, and then decreased after a longer time period; the theory that this behavior was due to degradation of the microspheres was confirmed by SEM.

Keywords Polyesteramide · Microspheres · Solvent evaporation method · Surface morphology

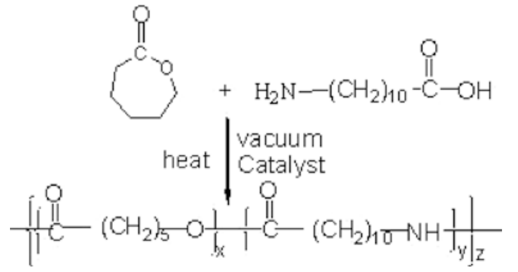
Introduction

Aliphatic polyesteramide copolymer is a promising biodegradable polymer due to its amphiphilicity and biodegradability [1, 2, 3, 4]. Its hydrophobic ester linkages are hydrolytically degradable, whereas its hydrophilic amide groups could improve both thermal and mechanical characteristics via intra- or inter-chain hydrogen bonding interactions. In our laboratory, several kinds of biodegradable polyesteramide copolymers have been prepared [5, 6, 7, 8]. The thermal properties and hydrolytic degradation behaviors of these copolymers have been discussed in detail. But the microsphere-forming properties of them have never been investigated. In this paper, polyesteramide microspheres are prepared by a simple oil-in-water (O/W) emulsion solvent evaporation method. The polyesteramide copolymer is synthesized from ϵ-caprolactone (ϵ-CL) and aminoundecanoic acid (AU) by the melt polycondensation method [5, 7].

Experimental

Materials

11-Aminoundecanoic acid was CP reagent; all the others were AR reagents. All of the materials were used as received, without any purification.



Scheme 1 Synthesis of P(CL/AU)x/y polyesteramide copolymers

Synthesis of polyesteramide copolymers

The P(CL/AU)x/y copolymers were synthesized from ϵ-caprolactone and 11-aminoundecanoic acid by the melt polycondensation method according to Scheme 1 [5, 7]. A typical P(CL/AU)60/40 copolymer was prepared as follows. ϵ-CL (17.1 g, 0.15 mol), AU (21 g, 0.104 mol), titanium dioxide (0.1 g), Irganox1010 (0.1 g), and tetrabutyl titanate (0.05 g) were added to the reaction vessel under a nitrogen atmosphere. The mixture was kept at 110 °C for 1 h. Later, the temperature was gradually elevated to 160 °C over 30 min. Then, the mixture was rapidly heated to 240 °C under vacuum for another 1 h. At the end, the resultant P(CL/AU)60/40 copolymer melt was poured out onto a steel plate.

Purification of PEEA copolymers

The resultant polyesteramide copolymers were first dissolved in AR grade trichloromethane (TCM), and reprecipitated from the filtrate using AR grade *n*-hexane. Then this mixture was filtered and vacuum dried to constant weight. The purified materials were kept in desiccators before use. The copolymers used in this paper are listed in Table 1.

Preparation of polyesteramide microspheres

P(CL/AU)60/40 copolymer was used to prepare the polyesteramide microspheres due to its great solubility in TCM. PEA microspheres were prepared by the simple oil-in-water (O/W) emulsion solvent evaporation technique. P(CL/AU)60/40 copolymer was first dissolved in 10 ml TCM. The resultant polymer solution was then added into 200 ml of aqueous polyvinyl alcohol (PVA) solution, with continuous stirring at a predetermined adding rate. The O/W emulsion was formed by extensive stirring with a four-blade stirrer for 3 h at a predetermined rate. After 3 h, the evaporation of organic solvent was complete. In some cases, vacuum was applied to increase the solvent evaporation rate. The obtained microspheres were washed with distilled water three times and kept in water before testing. The preparation parameters of the obtained microspheres are shown in Table 2.

Table 2 Parameters describing the preparation of the microspheres

Sample	Adding rate (ml/min)	$C_{polymer}$ (%W/V)	C_{PVA} (%W/V)	V_{stir} (rpm)	Vacuum?[a]
B-1	3	2.5	2.67	1200	No
B-2	3	1.25	2.67	1200	No
B-4	3	3.75	2.67	1200	No
C-1	3	2.5	4	1200	No
V-1	3	2.5	4	1200	Yes
J-6	0.33	2.5	4	1200	No
C-2	3	2.5	1.5	1200	No
D-3	3	2.5	4	1600	No
D-4	3	2.5	4	800	No

[a] Indicates whether or not vacuum was applied during the microsphere-forming process

Surface morphology

The surface morphologies of the polyesteramide microspheres were observed on an optical microscope and a SEM (AMRAY, America).

Determination of particle size distribution

Polyesteramide microspheres were first dispersed in distilled water using ultrasound dispersion, and then the particle size distribution was determined using a Malvern Mastersizer (Malvern 2000, Malvern Instruments, UK).

Fourier Transform IR spectroscopy

Fourier transform (FT) IR (KBr) spectra of the copolymers were taken with a NICOLET 200SXV IR spectrophotometer.

^{1}H Nuclear Magnetic Resonance spectroscopy

^{1}H NMR spectra (in $CDCl_3$) were recorded using a Bruker 300 spectrometer (Bruker, Germany) at 300 MHz using trimethylsilane as an internal reference standard.

In vitro degradation study

The polyesteramide microspheres were incubated in a bottle filled with phosphate buffer saline (PBS, pH = 7.2) at 37 °C. The samples were removed from the bottles at a predetermined time. Then the surface morphologies of the PEA microspheres were observed on the SEM (AMRAY, America) after sputtering with gold.

Table 1 Chemical composition and intrinsic viscosity of the samples used in this paper

Polymer code	Molar ratio of ϵ-CL/AU		ϵ-CL (%wt)		[η] (dL/g)	M_n
	In feed	Exp[a]	Theoretical[c]	Exp[d]		
P(CL/AU)40/60	38.9/61.1	n/a[b]	28.4	n/a[b]	0.61	n/a[b]
P(CL/AU)50/50	49.0/51.0	48.5/51.5	37.3	37.1	0.73	n/a
P(CL/AU)60/40	59.1/40.9	58.9/41.1	47.3	47.1	0.69	18738[a]

[a] Determined by ^{1}H-NMR; [b] it is not soluble in $CDCl_3$, so the ^{1}H-NMR was not determined; [c] calculated from the molar fraction of ϵ-CL/AU in feed; [d] calculated from the molar fraction of ϵ-CL/AU according to ^{1}H-NMR analysis

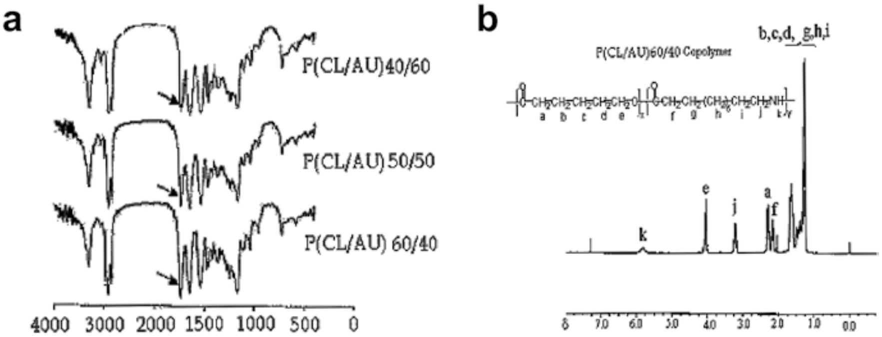


Fig. 1 a FTIR diagram of P(CL/AU)x/y copolymers. **b** H^1-NMR spectrum of P(CL/AU)60/40 ($CDCl_3$)

Results and discussion

Polymer characterization

FTIR spectra (KBr) of P(CL/AU)x/y copolymers are shown in Fig. 1a. The major characteristic absorptions identified in the FTIR spectra are: amide A (3315cm^{-1}) and amide B (3078cm^{-1}), amide I (1644cm^{-1}) and amide II (1540cm^{-1}), and aliphatic ester groups $\nu_{C=O}$ (1735cm^{-1}). Their relative intensities varied as expected with changes in the ϵ-CL/AU molar ratio. With increasing ϵ-CL content the absorption intensity at 1735 cm^{-1} increased. The typical ^{1}H-NMR spectrum of a P(CL/AU)60/40 copolymer is shown in Fig. 1b. The characteristic absorption peaks are indicated in this figure.

Preparation of polyesteramide microspheres

The solubilities of the P(CL/AU)x/y copolymers were greatly affected by their macromolecular structures, as shown in Table 3. With the increase in molar content of caprolactone, the solubility in $CHCl_3$ increased. P(CL/AU)40/60 was partly soluble in TCM, but P(CL/AU)50/50 and P(CL/AU)60/40 were easily soluble in TCM. In this paper, P(CL/AU)60/40 copolymer was selected to prepare microspheres in $CHCl_3$.

Table 3 Solubility of P(CL/AU)x/y copolymers

Solvent	P(CL/AU) 40/60	P(CL/AU) 50/50	P(CL/AU) 60/40
Trichloromethane	p.s	s	s
Dichloromethane	i	p.s	p.s
m-Cresol	s	s	s
Formic acid	p.s	s	s
Ethyl acetate	i	i	i
Methanol	i	i	i
Tetrahydrofuran	i	i	i
Acetone	i	i	i
Water	i	i	i

s: soluble, i: insoluble, p.s: partly soluble

There were several chemical and mechanical parameters that influenced the particle size distribution of the obtained microspheres [9, 10]. In this paper, the effects of stirring rate, PVA concentration, polymer solution concentration, polymer solution adding-rate, and vacuum on the microsphere-forming process were studied in detail.

Effect of stirring rate

The influence of stirring rate on particle size was very important. When the stirring rate increased, the microspheres became smaller and the size distribution decreased, as can be seen from Fig. 2 and Fig. 3.

Effect of PVA concentration

The existence of PVA emulsifier in the outer aqueous phase helps to reduce the interfacial tension between the inner organic phase and the outer aqueous phase. With the increase of PVA concentration in the outer aqueous phase, the interfacial tension reduced, so the obtained microsphere size decreased. The effect of PVA concentration on particle size distribution is shown in Fig. 4 and Fig. 5.

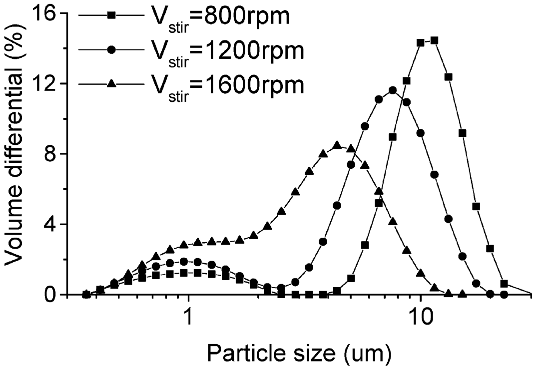
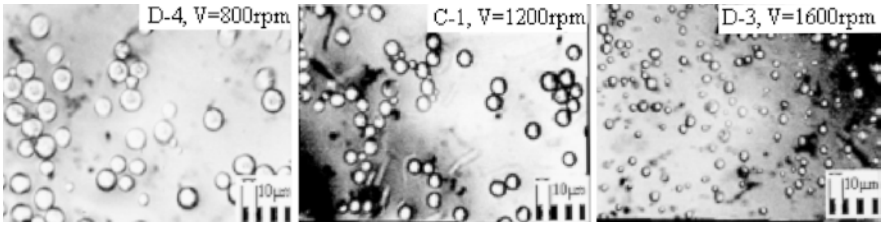


Fig. 2 Effect of stirring rate on particle size distribution

Fig. 3 Optical micrographs of microspheres prepared at various stirring rates



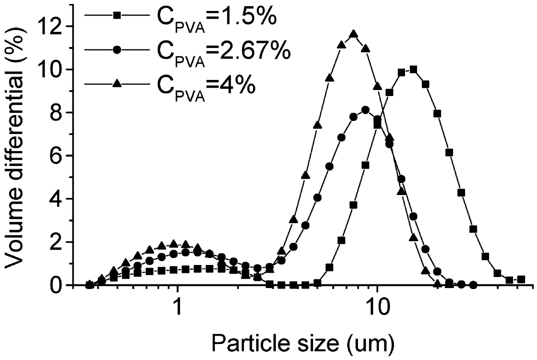


Fig. 4 Effect of PVA concentration on particle size distribution

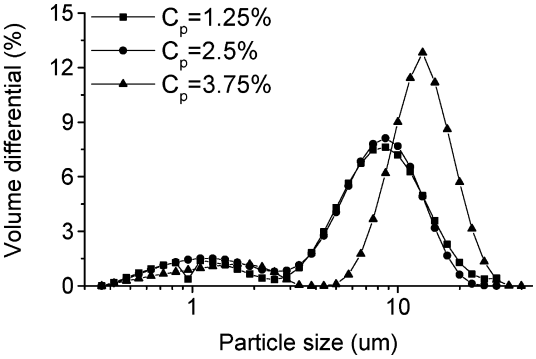


Fig. 6 Effect of P(CL/AU) copolymer concentration on particle size distribution

Effect of polymer solution concentration

Polymer concentration was a key factor influencing the particle size and distribution of PEA microspheres. For these studies, PVA was used as a stabilizer at a constant concentration of 2.67%w/v. PEA copolymer concentration was varied from 1.5 to 3.75%w/v. Figures 6 and 7 show that increasing polymer concentration lead to microspheres with a larger size and wider distribution. The increase in polymer concentration resulted in an increase in viscosity of the organic phase, which in turn lead to a reduction of stirring efficiency. The higher the viscosity of the polymer solution, the more difficult it was to form small emulsion droplets at the same input power of mixing. So, the obtained microspheres became larger with increasing in polymer concentration.

Effect of polymer solution adding-rate

Polymer solution-adding rate could have an effect on particle size and size distribution. When the polymer solution adding-rate decreases, the stirring efficiency increases, so the particle size and size distribution of PEA microspheres decreases. However, the change with adding-rate is not very marked, as can be seen from Fig. 8 and Fig. 9. As a result, the polymer solution-adding rate is a parameter of only minor influence. In this paper, PEA microspheres were prepared at a constant polymer solution-adding rate of 3 ml/min by a dropper.

Effect of whether vacuum was applied or not

When the vacuum was applied during the microsphere-forming process, the particle size and size distribution of PEA microspheres decreased. This can be seen from Fig. 10 and Fig. 11. This might be caused by the different solvent evaporation rates. When the vacuum was applied, the TCM evaporation rate increased, which resulted in PEA microspheres with a smaller particle size.

Fig. 5 Optical micrographs of microspheres prepared at various PVA concentrations

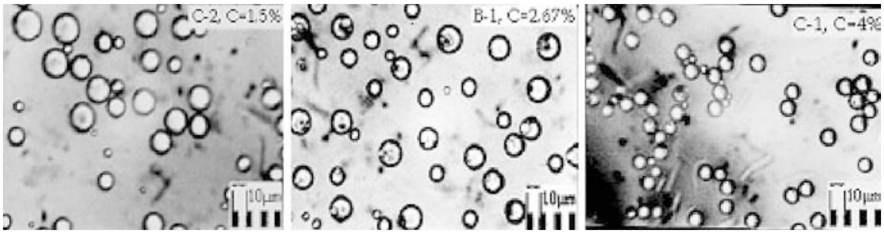
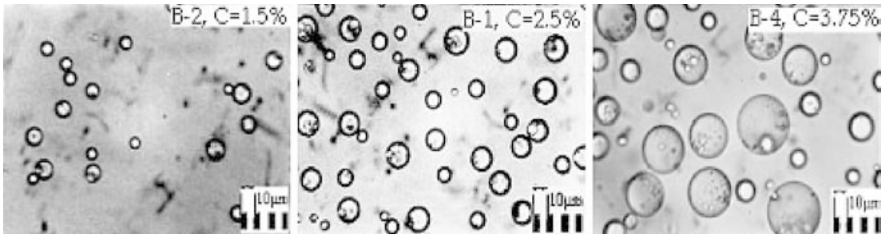


Fig. 7 Optical micrograph of PEA microspheres prepared at various polymer concentrations



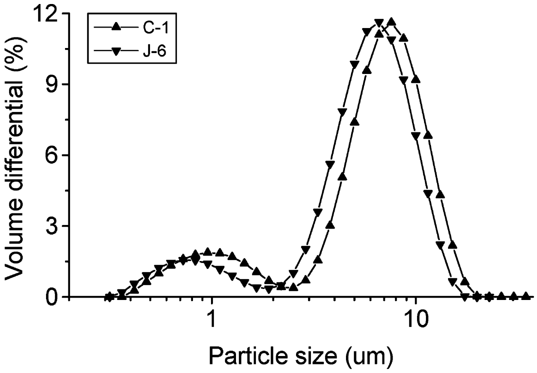


Fig. 8 Effect of polymer solution-adding rate on particle size distribution

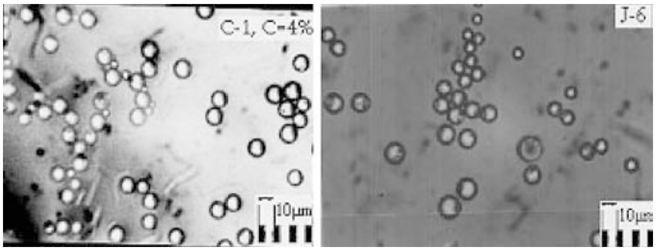


Fig. 9 Optical micrograph of PEA microspheres prepared at various polymer adding-rates

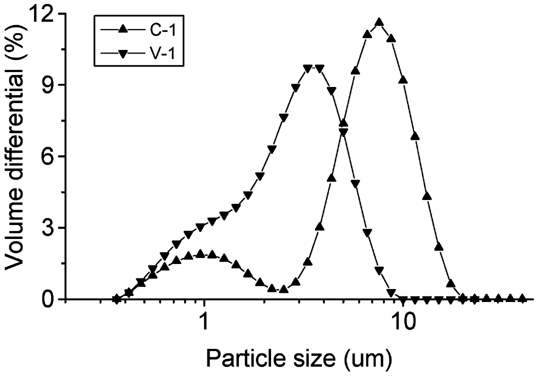


Fig. 10 Plot showing Effect of solvent evaporation rate on particle size distribution

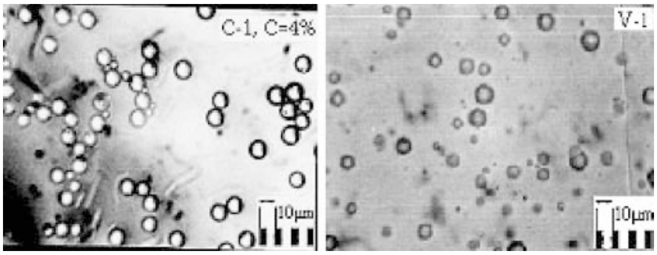


Fig. 11 Optical micrograph showing effect of solvent evaporation rate on particle size distribution

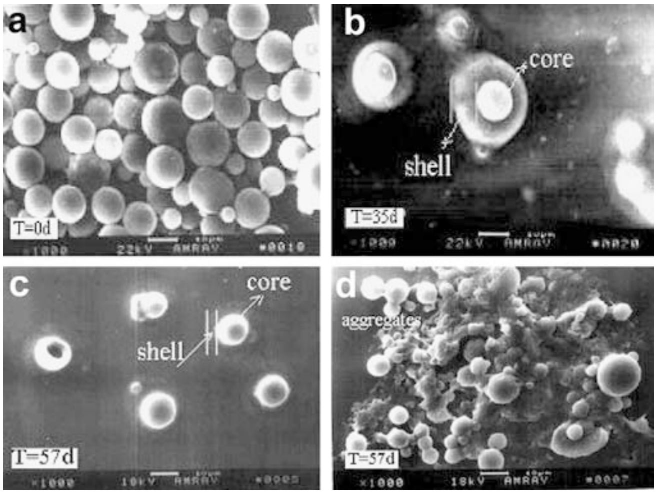


Fig. 12 Surface morphology of PEA microspheres after incubation in phosphate buffer saline (PBS) for various time periods (pH = 7.2, 37 °C, the bars represent 10 μm)

In vitro degradation behavior of PEA microspheres

PEA microspheres were incubated in phosphate buffer saline (PBS, pH = 7.2, 37 °C), and their degradation behavior was studied by SEM micrograph. These degradation results are shown in Fig. 12. After being incubated in PBS saline, the surface morphology of the PEA microspheres changed due to the hydrolytic degradation.

As shown in Fig. 12, the particle size of PEA microspheres increased at first because of the hydrophilic degradation product produced after incubation in PBS saline. At this time, the molecular weight of degradation products was not small enough to be soluble in aqueous medium, but they could swell to some extent. So the particle size of PEA microspheres increased. In Fig. 12b, the hydrophilic shell has come into being because of the hydrophilic degradation products. But when the degradation was extended to a relatively long period, the microspheres' hydrophilic shell became thinner, because the hydrophilic degradation products were dissolved in the aqueous medium due to their lower molecular weight, so the particle size decreased, as shown in Fig. 12c. Because of the great hydrophilicity, most of the microspheres were aggregated to form bigger aggregates, which can be seen from Fig. 12d.

The hydrolytic degradation behavior of P(CL/AU)x/y polyesteramide copolymers in PBS saline was studied in detail elsewhere [7]. For this kind of polyesteramide copolymer, when they were incubated in aqueous medium, the ester bond broke at first, and the hydrophilic oligomer was produced. As a result, the hydrophilicity of this copolymer increased with degradation time [7]. Therefore, this hydrolytic degradable copolymer might have a potential application in drug delivery systems.

Conclusions

P(CL/AU) polyesteramide copolymers were synthesized by a melt-polymerization method. Because P(CL/AU)60/40 could be dissolved in TCM, it was chosen for the preparation of microspheres by a simple O/W solvent evaporation method. Stirring rate, PVA concentration, PEA copolymer concentration, and vacuum were the most important preparation parameters that determined the microspheres' size distribution. In vitro degradation behavior of PEA microspheres in phosphate buffer saline was studied by SEM.

Acknowledgements This study was sponsored by the Outstanding Young Scientist Fund of Sichuan Province and the Hundreds of Talents Program of the Chinese Academy of Sciences.

References

1. Arvanitoyannis I, Nakayama A, Kawasaki N, Yamamoto N (1994) Angew Makrom Chem 222:111
2. Goodman I, Vachon RN (1984) Eur Polym J 20(6):529
3. Yujiang F, Kobayashi M, Kise H (2001) J Polym Sci A1 39:1318
4. Tuominen J, Seppälä JV (2000) Macromolecules 33:3530
5. Qian ZY, Li S, He Y, Li C, Liu XB (2003) Polym Degrad Stab 81:279
6. Qian ZY, Li S, Zhang H, Liu XB (2003) Colloid Polym Sci 281:869
7. Qian ZY, Li S, He Y, Zhang HL, Liu XB (2004) Biomaterials 25(11):1975 (DOI: 10.1016/S0142–9612(03)00604–5)
8. Qian ZY, Li S, He Y, Zhang HL, Liu XB (2003) Colloid Polym Sci 282(2):133 (DOI: 10.1007/s00396–003–0910–5)
9. Barbato F, Rotonda M IL, Maglio G, Palumbo R, Quaglia F (2001) Biomaterials 22:1371
10. O'Donnell PB, McGinity JW (1997) Adv Drug Delivery Rev 28:25